Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 39, No. 10, October 1982

Volume 39, n° 10, octobre 1982

Canada

Fisheries and Oceans | Pêches et Océans

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

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Ottawa K1A 0E6

Volume 39, No. 10, October 1982

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 39, No. 10, October 1982 Volume 39, n° 10, octobre 1982

Relationship Between Thickness of Daily Growth Increments in Sagittae and Change in Body Weight of Sockeye Salmon (*Oncorhynchus nerka*) Fry

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WILSON, K. H., AND P. A. LARKIN. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. Can. J. Fish. Aquat. Sci. 39: 1335 – 1339.

Sixty-four laboratory-reared sockeye salmon (*Oncorhynchus nerka*) fry were marked to enable identification as individuals. Each was weighed initially on June 6 or 8, 1979; and again on July 6; and surviving fish were weighed a third time on July 20. After the final weighing, sagittae were removed and a standard otolith radius, corresponding to each weight, was determined by counting back from the otolith edge the appropriate number of daily increments. The regression of ln otolith radius on ln fish weight was linear, with $r^2 = 0.92$, demonstrating a relationship between mean thickness of a daily increment in sagittae, and mean daily change in weight of the fry. Using this regression line, previous weights were back calculated from corresponding otolith radii with errors in the order of 15%.

Key words: otoliths, daily growth increments, back calculation, sockeye salmon

WILSON, K. H., AND P. A. LARKIN. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. Can. J. Fish. Aquat. Sci. 39: 1335 – 1339.

Soixante et quatre alevins de saumon nerka (*Oncorhynchus nerka*) élevés en laboratoire ont été marqués de façon à pouvoir les identifier individuellement. Le 6 ou le 8 juin 1979, le poids initial de chaque alevin a été déterminé; de nouveau le 6 juillet; et enfin, les survivants pesés une troisième fois le 20 juillet. Après la dernière pesée, nous avons enlevé les sagittae et, par comptage à partir du bord de l'otolithe du nombre approprié d'accroissements quotidiens, déterminé un rayon otolithaire standard correspondant à chaque poids. La régression de $\log_e$ du rayon de l'otolithe sur $\log_e$ du poids du poisson est linéaire, $r^2 = 0{,}92$, ce qui démontre une relation entre l'épaisseur moyenne d'un accroissement quotidien dans les sagittae et le changement moyen quotidien du poids des alevins. Utilisant cette ligne de régression, nous avons rétrocalculé les poids antérieurs à partir de rayons otolithaires correspondants, avec erreurs de l'ordre de 15 %.

Received September 18, 1981
Accepted June 16, 1982

Reçu le 18 septembre 1981
Accepté le 16 juin 1982

Printed in Canada (J6640)
Imprimé au Canada (J6640)

OTOLITHS have been used as a method of aging fish since the turn of this century (Graham 1929), and a recent study examined the relationship between fish growth and otolith size in salmonids (Jonsson and Stenseth 1978). Until recently, only techniques utilizing annual marks have been available for age and growth studies. The presence of daily growth increments in teleost otoliths (Panella 1971), including those of sockeye salmon (*Oncorhynchus nerka*) (Wilson and Larkin 1980), provides an important new methodology for age and growth studies, particularly in early life history stages.

The purpose of the experiment here reported was to examine the relationship between fish weight and otolith size over short-time intervals for sockeye salmon fry, utilizing daily increments as the time marker.

Materials and Methods

Sockeye salmon fry were reared in the laboratory from fertilized eggs taken in the fall of 1978 at the Weaver Creek spawning channel near Mission, British Columbia. A random sample of 64 fry was branded to enable identification of individuals. The marks consisted of the presence or absence of a cold brand in one or more of five locations on the body. The fry were anesthetized with MS−222 and branded with a copper wire immersed in liquid nitrogen.

Each of the fry was weighed and branded before being placed in a tank (30 × 30 × 60 cm). One tank was stocked on June 6, 1979; the other 2 d later. The tanks were maintained on an 18-h dark, 8-h light photoperiod. A flow of ~ 2 L/min of dechlorinated tap water was maintained in each tank. The temperature was not controlled; the mean daily temperature increased from 6 to 13°C during the course of the experiment.

The fish were not fed for 24 h prior to weighing to minimize variation in weight related to stomach contents; otherwise, the fish were fed at 1-h intervals eight times a day commencing at 08:00. Weighings of fish were all carried out during the same period of the day, during the late afternoon. Each fish was weighed twice, the two weights compared to ensure agreement within 0.01 g, and averaged.

Thirty days after the first tank was stocked, the fish in both tanks were anesthetized and reweighed. Fry surviving the second weighing were reared for an additional 2 wk, when excessive mortality forced an end to the experiment. Data were recorded for 22 fry which died as a result of handling stress during the second weighing, as well as for 14 fry remaining alive at the end of the experiment. Those fish which died during the course of the experiment (13 during the 1st month and 15 during the final 2 wk) were discarded from the analysis for the period in which they died.

Sagittae were removed and ground on both sides using the method of Neilson and Geen (1981). Rapid grinding was performed on 30-μm paper and finishing with Mylar lapping film (0.3 μm). After polishing one side of the otolith, the acrylic glue was softened by immersing the slide in acetone, the ground surface of the otolith was reattached to the slide, and the otolith reground. The otoliths of seven fish were crystalline, broken, or otherwise unreadable, and were omitted from further analysis.

One otolith from each fish was photographed using Kodak Panatomic-X film in a 35-mm camera mounted on a compound microscrope. Projector slides were prepared directly from film negatives. Slides were projected at a fixed height and angle, with a fixed focus, to achieve a magnification of 382×. Measurements were taken directly from the projected image.

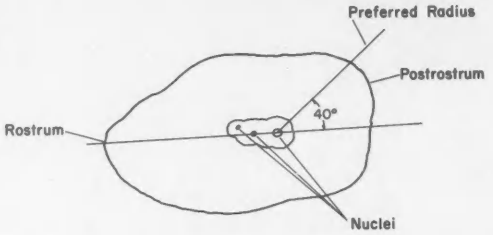

FIG. 1. Sagitta of an *O. nerka* fry showing rostrum, postrostrum, nuclei, and location of preferred radius for measurement.

In sockeye salmon the sagitta (Fig. 1) typically has several nuclei and changes shape as it grows. The sagitta of the yolk sac fry is oval in shape, but as the fry grows the sagitta becomes heart-shaped with the rostrum and postrostrum exhibiting the most rapid radial growth. These areas of greatest growth usually show the most distinct increments. The postrostrum in our samples was less prone to breakage during preparation, more uniformly shaped, and consistently had the most easily discernible daily growth increments.

To ensure that measurements were taken on the same radius for each otolith, a line was drawn to the rostrum through the nucleus immediately adjacent to the postrostrum. A radius was drawn in a straight line from this nucleus through the postrostrum at a fixed angle of 40° (Fig. 1).

The growth increment in each otolith corresponding to the date of each weighing was determined by counting back the appropriate number of daily increments from the edge and measuring along the radius. Figure 2 shows three views of the same otolith showing the location of the measurement radius and the increment count. The otolith radius corresponding to the weight as measured 28 d prior to death would be measured to the 28th increment from the edge (marked with an arrow).

Each radius was measured three times and average values calculated. Thus, a series of two or three weights and a standard otolith radius corresponding to each weight were determined for each fish.

Results

The relationship between otolith measurements and weights of fry was evidently curvilinear. Accordingly, a standard regression line was fitted to the log of the weight and the log of the radius. Additionally, the data in raw form were fitted by orthogonal polynomials.

The orthogonal polynomial regression yielded an $r^2 = 0.9157$, but the residuals showed a marked nonrandom trend in that the weights of the fish with the smallest radii were substantially underestimated.

The standard linear regression for the logged data yielded a line of best fit $\ln W = -11.27 + 4.409 \ln R$ and an r^2 of

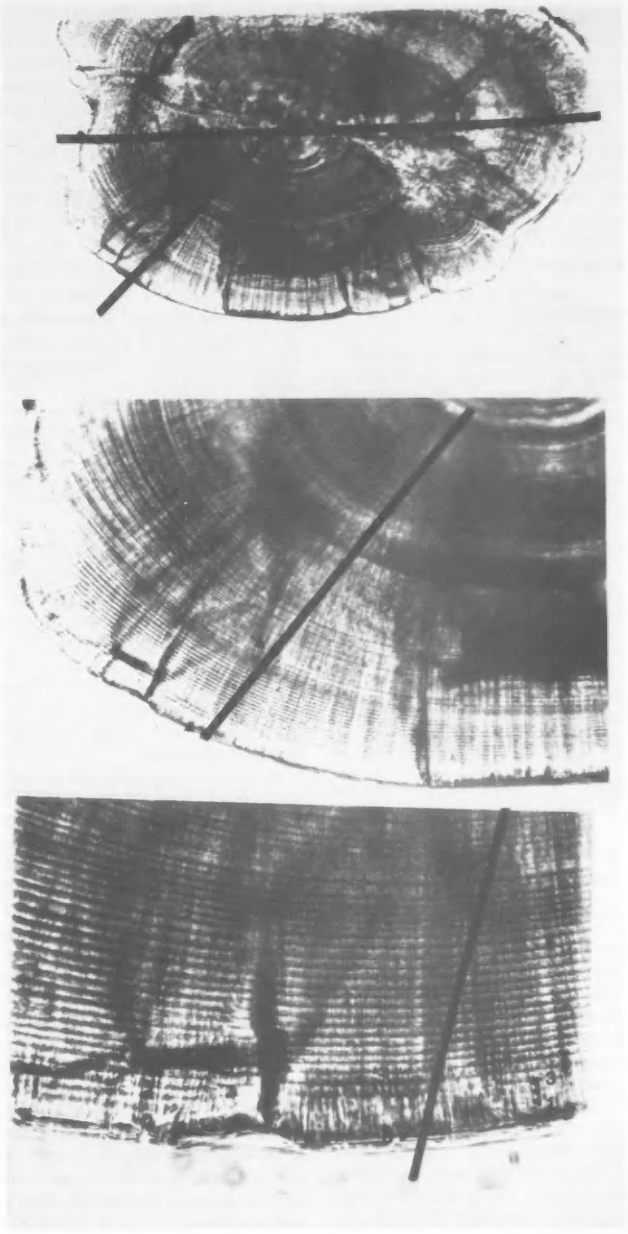

FIG. 2. Sagitta of a fry that had been weighed 28 d previously. *Upper* figure, whole otolith; *middle* figure, at radius of measurement; *lower* figure, at edge. In each case the ring 28 in from the edge is indicated by a small black arrow.

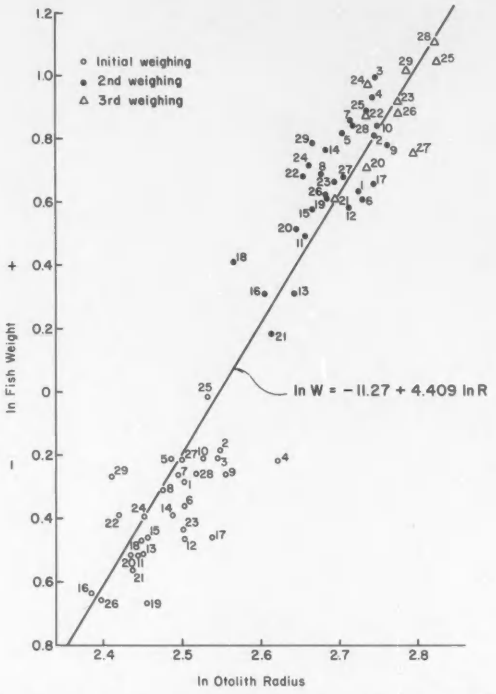

FIG. 3. Natural logarithm of fish weight and ln otolith radius for 29 individual *O. nerka* fry at an initial (June 6 or 8), second (July 2), and third (July 20) weighing.

0.9230 (Fig. 3). Although the data points for each fish are linked, the conservative assumption was made that all data points were independent.

The weight of each individual at the time of previous weighings was back calculated using the formula

$$\ln W_1 = \ln W_2 - b(\ln R_1 - \ln R_2)$$

where W_1 is the weight to be estimated, W_2 is some later measured weight, R_1 is the radius corresponding to the weight to be estimated, R_2 is the radius corresponding to W_2, and b is the slope as estimated by regression (= 4.409).

This procedure of back calculation is readily derived from the formula for the linear regression, making the assumption that the slope is constant, and all variation among individuals is reflected in variation of the intercept.

The differences between the weights observed and the weights predicted by back calculation (i.e. the residuals) were not distributed at random (Fig. 4). At the lowest and highest weights the residuals were negative, the procedure of back calculation underestimating. In the midrange of weights the residuals were positive, indicating overestimation.

Discussion

Because the radius measurements were determined primarily on the basis of daily growth increments, the close relationship between otolith radius and weight shows a strong relationship between the mean width of a daily growth increment in sockeye salmon fry sagittae, and a mean daily change in the weight of the fry.

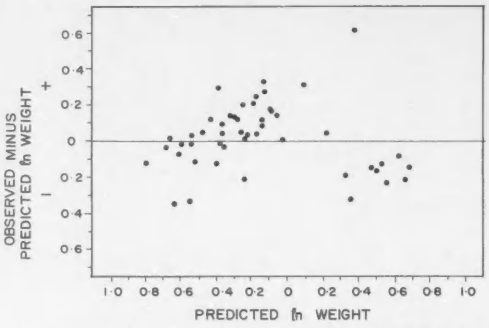

FIG. 4. Residuals of back-calculated ln weight (ln observed fish weight minus ln predicted fish weight plotted against predicted ln weight.

There are several possible biological bases for departure of the relationship from a linear log form, as indicated by the nonrandom distribution of residuals. Abrupt changes may occur in the pattern of otolith growth or body weight. The otolith is presumably growing in a comparable fashion to other hard parts, and an inflexion might occur after an initial period of more rapid growth. Change in weight, used here because it is more readily measured in small fish, may also be subject to relatively abrupt change in rate either in response to developmental change or as a result of stress.

Another possible cause of the pattern of departure of observed from predicted values could be selective mortality in the course of the experiment. If smaller fish tend to remain relatively smaller and larger fish tend to remain relatively larger, then size-selective mortality at the second weighing could have contributed not only to the relatively large value of the slope, but also to the pattern of departures of observed from back-calculated sizes. This does not appear to have been a factor in this analysis because the mean initial weight of those fish which died at the second weighing (0.693 g) was not significantly less than the mean initial weight of the fish which survived to the end of the experiment (0.700 g).

Measurement error may be a factor because the statistical procedure of back calculation assumes a constant slope. If measurement error or other random effects are a significant component in the variation of weight and radius, this method of back calculation will always tend to overestimate the previous weight of fish with a large weight-to-radius ratio and underestimate those with a small ratio. The back-calculation formula assumes that the fish maintain a constant rank order with respect to the weight and otolith radius relationship. By connecting the points representing the weights and corresponding radius measurements for the individual fish (as shown in Fig. 3), it is apparent that changes in rank order did occur.

Error in measuring fish weight would appear to be a minor

contributor to the uncertainty in the relationship between fish weight and otolith radius. Measurement error is, however, a major component of the variation in radius values. Errors of ±5% over three measurements of a radius were not uncommon. Much of the variation resulted from difficulty in determining the exact location of the appropriate nucleus. Other technical problems contributed to variation in the radius measurement; chipping or cracking of the otolith during grinding or preparation could obscure or shift the apparent location of the rostrum or postrostrum; membranes adhering to the otolith could obscure the otolith, and variation in the amount of material removed during grinding could change apparent ring width.

Variation in the shape of the otolith with size and between individuals of the same size also introduces variation in standard radius measurements, particularly when a relatively simple standard method is used. For example, because the otolith changes shape as it grows, a straight line drawn from the nucleus to the outside edge of the otolith will not necessarily be perpendicular to any or all of the rings it intersects, nor will it necessarily intersect all of the rings at the same angle. The use of a curved radius, perpendicular to each ring it intersects, was considered. Unfortunately, the length of a curved line proved difficult to measure accurately and directly, and the resulting radius did not always leave the nucleus at the same angle to the longitudinal axis. Complex age, size, and shape specific measurement systems would almost certainly reduce variation in radius measurement, as would improved techniques of otolith preparation.

Despite these several considerations, it is to be emphasized that the relationship between fish weight and otolith radius (as determined by daily increments) provides a satisfactory method for back-calculating fish weight at previous ages. The errors that arise from the techniques used here are relatively small (of the order of 15%) and permissible in prediction even over as short a period as 2 wk. It is thus possible to use the technique of back calculation on otoliths for gaining better understanding of early life history events of Pacific salmon. For example, it is commonly speculated that rapid initial growth is critical to survival or that various environmental changes have their most significant effects at early growth stages. These and other questions related to the first months of growth should be assisted substantially by the knowledge that the daily growth increments are proportional in thickness, on the average, to the increments in body weight.

Acknowledgments

We thank Gail Sugden and Moira Greaven for their excellent technical assistance, and gratefully acknowledge financial support of the Marine Resources Branch, B.C. Ministry of Environment; and the Natural Sciences and Engineering Research Council of Canada. The International Pacific Salmon Fisheries Commission also provided assistance.

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Otoliths of Chinook Salmon (*Oncorhynchus tshawytscha*): Daily Growth Increments and Factors Influencing Their Production

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NEILSON, J. D., AND G. H. GEEN. 1982. Otoliths of chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Can. J. Fish. Aquat. Sci. 39: 1340 – 1347.

The effects of photoperiod, feeding frequency, and water temperature on formation of otolith daily growth increments in juvenile chinook salmon (*Oncorhynchus tshawytscha*) were examined. Feeding frequency influenced both increment number and width, whereas photoperiod and temperature affected only increment width. Fish fed once/24 h produced one increment every 24 h on average, while fish fed 4 times/24 h produced more than one increment every 24 h. Wider increments were produced in fish exposed to warmer water (11°C) or 24 h of darkness. The ratio of otolith size to fish size remained constant throughout and between the photoperiod, temperature, and feeding frequency experiments, regardless of the number or width of increments produced. Although otolith growth is isometric with respect to increase in fish length under these experimental regimes, otolith microstructure will differ in fish of the same size reared under different environmental conditions. An understanding of factors affecting otolith increment production is required before increment number and width can be used to assess growth rates.

Key words: otolith, daily growth increments, chinook salmon

NEILSON, J. D., AND G. H. GEEN. 1982. Otoliths of chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Can. J. Fish. Aquat. Sci. 39: 1340 – 1347.

Les effets de la photopériode, de la fréquence des repas et de la température de l'eau sur la formation quotidienne des zones de croissance de l'otolithe de jeunes saumons chinook (*Oncorhynchus tshawytscha*) ont été étudiés. La fréquence des repas influence à la fois le nombre et la largeur des zones de croissance de l'otolithe, alors que la photopériode et la température n'ont d'effet que sur leur largeur. Les poissons nourris une fois par 24 h produisent en moyenne une zone de croissance par 24 h, alors que ceux nourris 4 fois par 24 h produisent plus d'une zone de croissance pendant la même période. Les poissons exposés à de l'eau plus chaude (11°C) ou à 24 h de noirceur produisent des zones de croissance plus larges. Le rapport de la taille de l'otolithe à celle du poisson demeure constant pendant ou entre les photopériodes, de même que pendant ou entre les expériences de température et de fréquence des repas, quel que soit le nombre ou la largeur des zones de croissance produites. Bien que la croissance de l'otolithe soit isométrique par rapport à l'augmentation de longueur du poisson dans ces conditions expérimentales, la microstructure de l'otolithe diffère chez des poissons de même taille élevés dans des conditions ambiantes différentes. Avant de pouvoir utiliser le nombre et la largeur des zones de croissance de l'otolithe pour évaluer les taux de croissance, on devra avoir une bonne compréhension des facteurs influençant la production des zones de croissance de l'otolithe.

Received January 15, 1982
Accepted June 30, 1982

Reçu le 15 janvier 1982
Accepté le 30 juin 1982

SINCE Pannella (1971) described daily growth increments in marine fish otoliths, research on otolith microstructure has proliferated. Daily growth increments have been reported in several species of fish, usually juveniles less than 200 d old. Counts of daily growth increments were correlated with the age of the fish in days after hatching (Pannella 1971; Brothers et al. 1976; and others). As well as permitting improved resolution of age, otolith microstructure allows refined estimates of fish growth (Struhsaker and Uchiyama 1976; Methot and Kramer 1979; Wilson and Larkin 1982).

The validation of conclusions based on otolith microstructure requires data on the effects of environmental variables such as light, temperature, and feeding frequency on otolith increment formation. Recent evidence indicates, for

Printed in Canada (J6767)
Imprimé au Canada (J6767)

example, that more than one growth increment may be formed each day under certain environmental conditions (Brothers 1979; Pannella 1980). Several environmental factors have been implicated in the formation of daily growth increments. Pannella (1980) suggested a close relationship between feeding frequency and number of growth increments formed, although no supporting data were offered. Taubert and Coble (1977) stated that a 24-h light/dark (LD) cycle entrained a daily periodicity in increment formation, while Brothers (1979) suggested that diel changes in water temperature may act as a zeitgeber. Campana and Neilson (1982) found that the number of increments produced by starry flounders (*Platichthys stellatus*) was unaffected by photoperiod or temperature fluctuations. To date, research has largely been confined to the study of effects of environmental variables on numbers of otolith growth increments produced. Few authors have attempted to relate otolith increment width to environmental variables. In this paper, we examine the effects of feeding frequency, photoperiod, and constant temperature regimes on the number and width of otolith growth increments formed in juvenile chinook salmon (*Oncorhynchus tshawytscha*).

Materials and Methods

Feeding Frequency

We used chinook salmon fry from the 1979 brood of the Canada Department of Fisheries and Oceans Capilano Salmon Hatchery, Vancouver, British Columbia. During the period from fertilization through yolk sac absorption, fish were held in Heath trays in a darkened room at an average of 4°C. The fish were transferred to Simon Fraser University ~ 90 d after hatching (free-swimming fry) and held in 28-L flow-through aquaria supplied with aerated and dechlorinated water. Fish were not fed prior to transfer, as yolk sacs were present until that time.

For experimental purposes, two groups of similarly sized fish were held under a 12:12 LD light regime for 65 d. One group of 100 fish was provided with excess food (~ 2.5 g Oregon Moist Pellets per feeding) by an automatic fish feeder at 6-h intervals. Excess food was siphoned from the aquarium at 13:00 each day. A second group of 100 fish was provided with ~ 10 g at 12:00 every day, with excess food removed after 30 min. During these experiments water temperature ranged from 10 to 12°C and did not vary in any consistent diel fashion.

Temperature

Fish incubated at the Capilano Salmon Hatchery (1979 brood) were transferred as free-swimming fry 45 d after hatching to two outside holding tanks on hatchery premises. Prior to transfer to the holding tanks, the fish had previously been kept in a darkened Heath tray at 4°C until hatching, and at 12°C from the hatching to the free-swimming stage.

The experimental fish in the holding tanks were held at mean temperatures of 5.2 ± 1.6°C (±1 SD, range 2−9°C) or 11 ± 1.4°C (range 7−13°C). Although no diel fluctuations in water temperature were noted, mean daily temperature increased gradually over the 45-d duration of the experiment. Fish were fed to excess once every day.

Photoperiod

The role of photoperiod in modifying otolith growth increments at the earliest stages of development was examined. Two groups of 100 unfertilized eggs (1980 brood) were transported from Capilano Hatchery to Simon Fraser University and fertilized. One lot was placed in a darkened Weiss-type incubation funnel while a second lot was placed in a similar funnel and exposed to a 12:12 LD photoperiod. The water temperature through the incubation period (fertilization to yolk sac absorption) ranged from 7 to 9°C and did not vary in any consistent diel fashion. The experiment was terminated 80 d after fertilization (30 d after hatching).

The role of photoperiod in otolith increment formation in older fish was examined by holding a group of 90-d-old fry (1979 brood, Capilano Salmon Hatchery) under constant illumination from a fluorescent light source (200 lx) for 65 d. A second group of fry was held under a 12:12 LD photoperiod for 65 d. Light intensities were 200 and 40 lx during the light and dark periods, respectively. Both groups were fed ~ 2.5 g of Oregon Moist Pellets every 6 h. Water temperature ranged from 10 to 12°C and did not fluctuate with diel periodicity.

Preparation and Examination of Otoliths

The sagittae, the largest of the three pairs of otoliths in salmonids, were dissected from fish preserved in 95% ethanol and stored dry in individual wells of tissue culture plates until further processing. To prepare the otolith for microscopic examination, it was attached with the sulcus acusticus (a prominent depression on the proximal surface), side down to a standard glass microscope slide with "Crystalbond" (distributed by Aremco Ltd., New York), a thermosetting plastic resin. The otolith was ground and polished on its sagittal surface using a grinding jig (Neilson and Geen 1981) and metallurgical lapping films whose grit size ranged from 0.3 to 30 μm. If the specimen was too opaque to allow examination using transmitted light microscopy after the first grinding, the slide was gently heated, and the otolith removed and reset in the thermosetting plastic with the ground surface against the slide. The proximal side of the otolith was then ground and polished to reduce the thickness of the preparation.

If the otolith was to be examined using the scanning electron microscope (SEM), it was transferred to a SEM specimen stud, etched by immersing the stud and otolith in a 1% HCl bath for 90 s, and then rinsed with water. The weak acid differentially etches various parts of the otolith, resulting in the growth increments having a three-dimensional structure, a prerequisite for scanning electron-microscopic examination. After etching, the regularly recurring increments are bipartite, consisting of relatively narrow, deeply etched zones and relatively wide, lightly etched zones. After drying, the stud and otolith were placed in a planetary specimen holder and transferred to a vacuum evaporator where the otolith was gold-plated. Otoliths were viewed using a Perkin−Elmer Autoscan SEM at 600−900× magnification.

Some otoliths, mainly those from the temperature experi-

ment, were examined with a research quality light microscope at 800−1200× magnification. Light microscopy was most appropriate when examining otoliths from small fish (<50 d after hatching) as sufficient light was transmitted through the relatively thin otolith for examination.

Scanning electron and light micrographs were attached to a Tektronix 4954 digitizing table on which increment widths were measured. The digitizing table had a direct link to computer facilities where data were stored and analyzed further using standard statistical programs. The resolution of the digitizing table was 0.3 mm, representing about 3% of the average photographic enlargement of a 2-μm increment. Using a simple computer program, the data were transformed from scalar distances defined by increments along a radius of the otolith to increment widths. We measured increment width along a radius 30° from the long axis of the otolith in the quadrant of maximum growth, as suggested by Pannella (1980). The increments were typically widest along this radius.

We determined which increments were to be digitized by locating checks in the otherwise regular pattern of increment deposition caused by events such as hatching, transport stress, or abrupt temperature shifts. Such checks were common to all fish examined. After confirming that the checks corresponded to an event of known date, they were used as dated references for counts or increment width measurements.

Error of the digitizing table operator in recognizing and recording increment boundaries was assessed by digitizing a series of 50 increments on four duplicate SEM micrographs. The mean increment widths for each of the four trials did not significantly differ ($P > 0.05$), and the same number of increments was recorded.

Measurements of increment width might be affected by any deviation in the angle of grinding and polishing from the preferred plane (the plane of maximum area on a longitudinal axis). We simulated this effect by tilting the SEM stage through 15°, which was assumed to be the maximum deviation of the grinding plane. Micrographs of an SEM preparation were taken at 5° intervals through this arc, and digitized. The error in measurement of otolith increment width through this arc was <1% in the 600−900× magnification range used, and is not considered a serious source of error.

The results obtained using light and scanning electron microscopy were compared by making a reference mark on five otoliths with a carbide-tipped stylus. This mark, visible under light and scanning electron microscopes, served as the starting point for counts and increment width measurements. Counts and increment widths obtained using either type of microscope did not differ significantly ($P > 0.05$). Thus, etching the otolith surface with weak acid prior to SEM examination did not affect increment width or number.

Fifty pairs of otoliths from alevins were examined to determine whether counts or otolith increment widths varied according to the side of the fish from which the otolith originated. Using a Wilcoxon paired sample test, we detected no difference in counts obtained from either side of the fish ($P > 0.05$). However, otoliths removed from the left side of the fish were significantly larger (maximum length) than those from the right side. Data reported here are from left-side otoliths only.

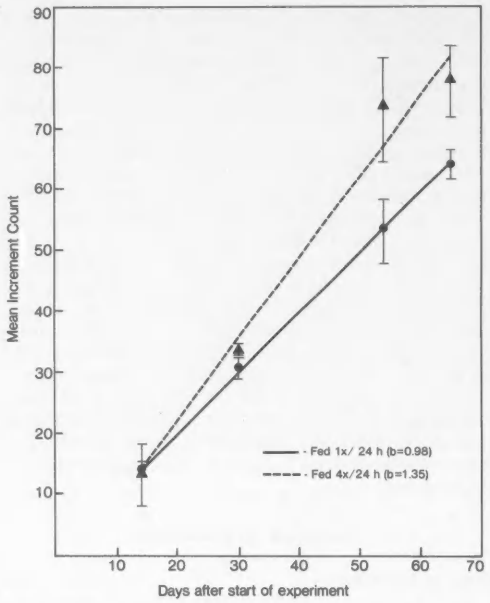

FIG. 1. Relationship of number of otolith increments formed in chinook salmon fry fed once/24 h or 4 times/24 h. Points on the regressions are means of counts from 5 to 10 otoliths (one feeding/24 h) and 4 to 14 otoliths (four feedings/24 h), and 1 SD is indicated by the error bars.

Results

FEEDING PERIODICITY

Fish fed once daily deposited an average of one growth increment per 24 h, whereas fish fed 4 times/24 h formed more than one growth increment per day (Fig. 1, 2). The slopes of the two regressions (0.98 and 1.35 for one feeding/24 h and four feedings/24 h, respectively) are significantly different (*t*-test, $P < 0.05$). The slope of the regression for one feeding/24 h does not significantly differ from unity ($P > 0.05$), indicating that one increment was formed every 24 h. We inferred that total food assimilated by fish in the two groups was comparable as there were no significant differences in fork length at the conclusion of the experiment (analysis of variance, $P > 0.05$).

Mean increment widths during the 14- to 30-d period of the experiment were 1.52 ± 0.10 and 1.30 ± 0.09 μm for fish fed once and four times every 24 h. During the 31- to 54-d period, mean increment widths were 2.27 ± 0.39 and 1.25 ± 0.25 μm for one feeding/24 h and four feedings/24 h, respectively (Fig. 3). Mean increment widths were significantly different during both periods (analysis of variance, $P < 0.05$ and < 0.01 for days 14−30 and 31−54, respectively).

The slopes of regressions of otolith size on fish size did not significantly differ for each feeding regime, nor were they different from the slope of the regression representing pooled

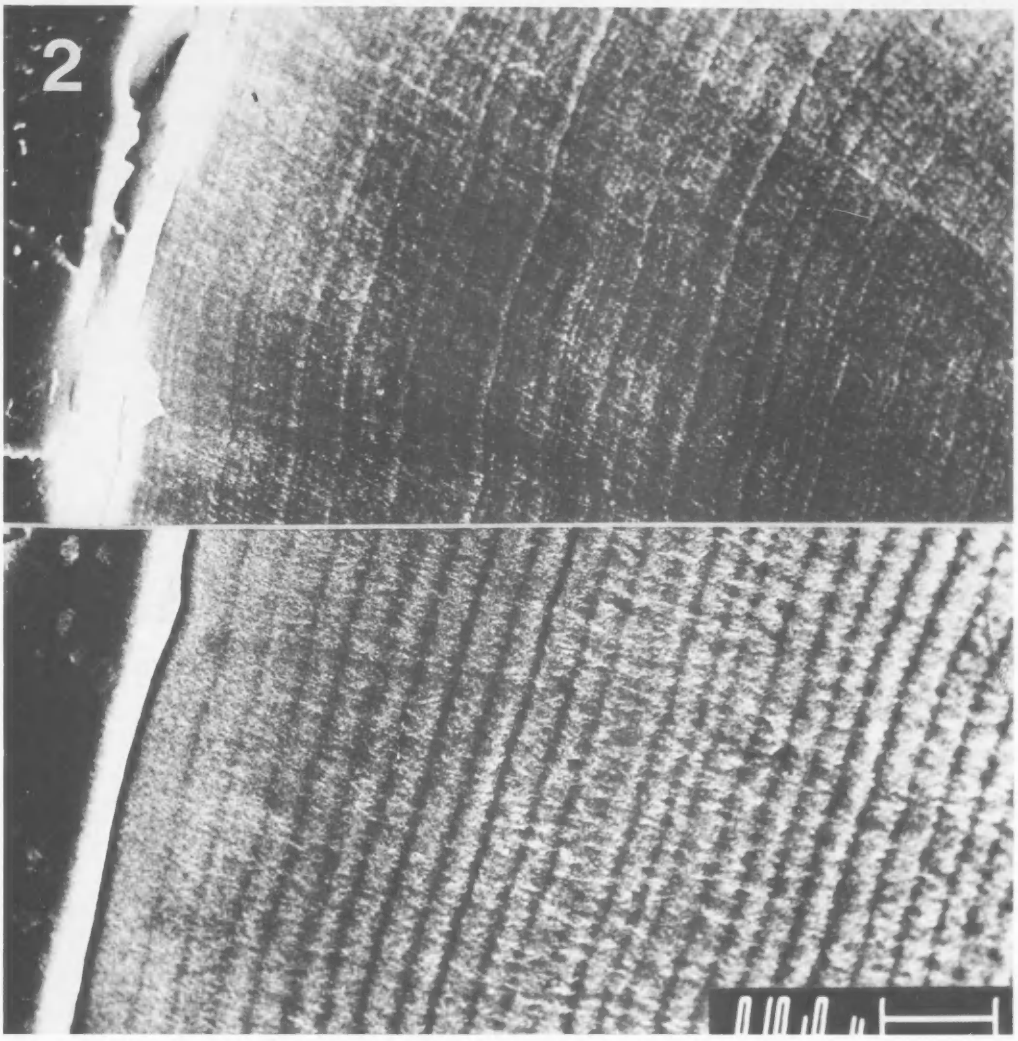

FIG. 2. Scanning electron micrographs of ground and etched chinook salmon otoliths taken from fry fed 4 times/24 h (*top*) or once/24 h (*bottom*). Fish were sacrificed at day 65 of the feeding frequency experiment.

data from all experimental treatments (Fig. 4, analysis of covariance, $P > 0.05$). Because both groups of fish grew at the same rate, and the size of the otolith relative to fork length remained the same, the increase in otolith size results from deposition of fewer, wide increments (one feeding/24 h) or more, narrow increments (four feedings/24 h).

TEMPERATURE

The mean number of increments produced in free-swimming fry reared under the warmer and colder temperatures for 45 d were 42.50 ± 4.87 and 42.40 ± 3.57, respectively, and were not significantly different (analysis of variance, $P > 0.05$, n = 10). Mean width of increments formed during this 45-d period were 1.54 ± 0.32 μm (n = 10) and 1.11 ± 0.12 μm (n = 10) for fish reared in warmer and colder water, respectively, and were significantly different (analysis of variance, $P < 0.05$). At the end of the 45-d period, mean fork lengths of fish were 39.0 ± 1.2 mm and 53.2 ± 4.5 mm under the lower and higher temperatures,

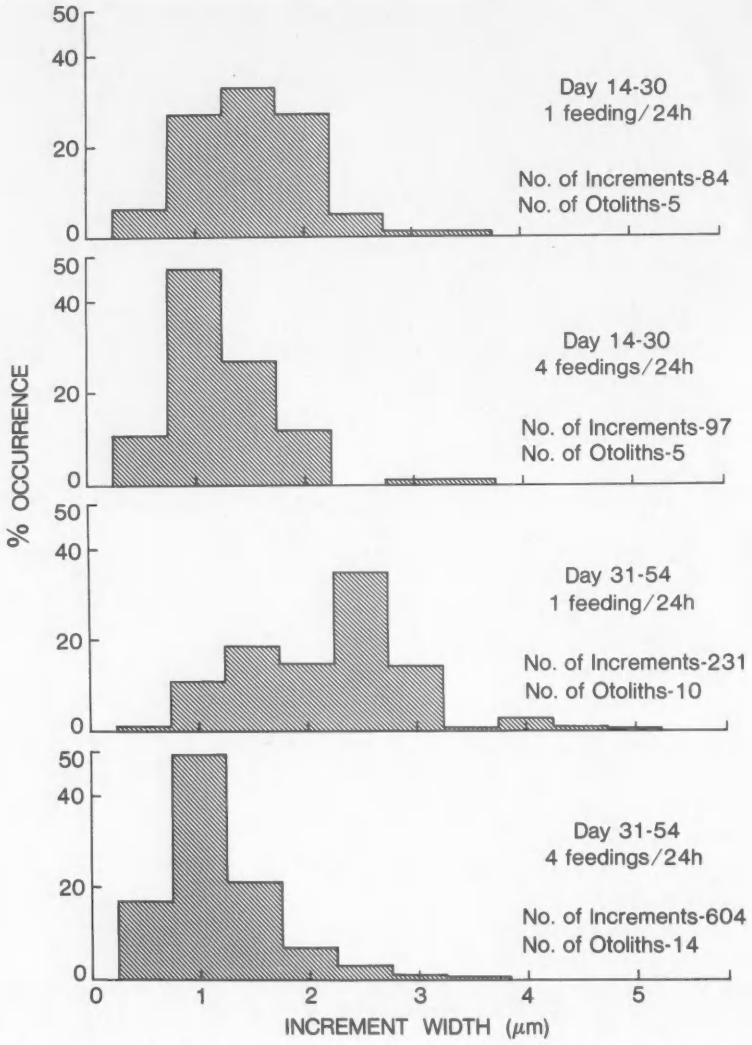

FIG. 3. Distribution of chinook salmon fry otolith increment widths through days 14–30 and days 31–54 of the feeding frequency experiment.

respectively, and were significantly different ($P < 0.01$). Slopes of regressions of otolith size on fish size did not significantly differ (analysis of covariance, $P > 0.05$, (Fig. 4) reflecting isometric increases in otolith size and fish length. In this instance age in days was reflected by the number of increments and growth rate indicated by increment width.

PHOTOPERIOD

Photoperiod did not influence the numbers of increments produced by fish during the alevin stage. Slopes of regressions of mean increment counts on days after start of treatment did not differ significantly between treatments, or from unity (t-test, $P > 0.05$). This indicates that in fish feeding endogenously, one growth increment was formed on average every 24 h, regardless of photoperiod.

Although increment widths were not examined in this phase of the work, the mean total length of otoliths of fish held under 24 h of darkness was significantly greater (analysis of variance, $P < 0.01$, n = 12) than that of fish held under a

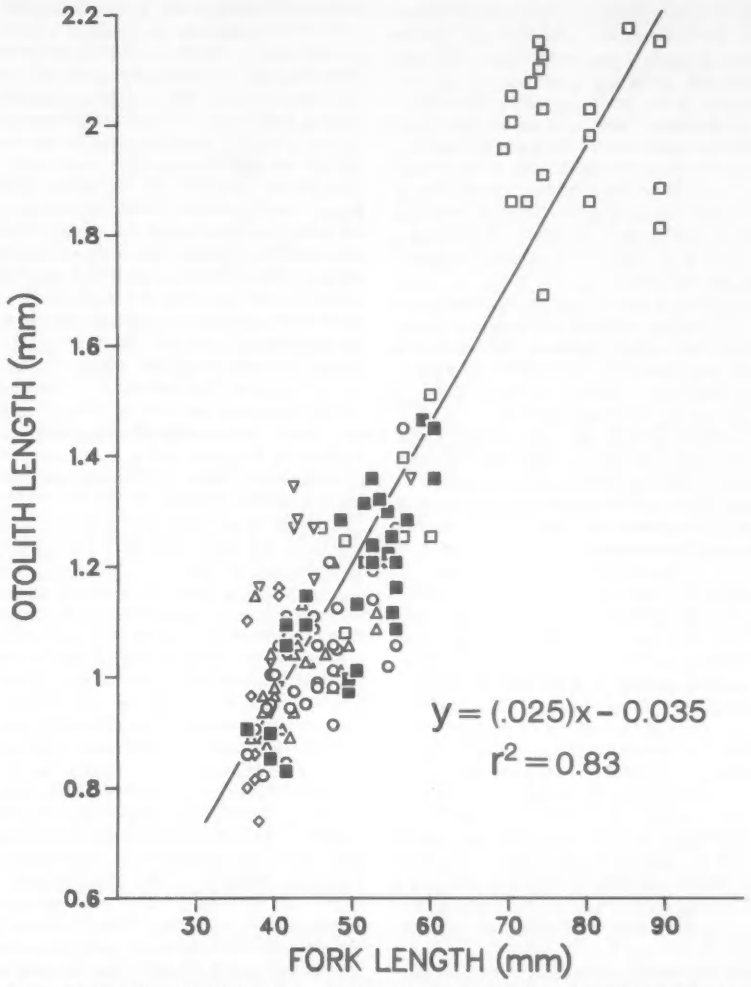

FIG. 4. Relationship of otolith length versus fish length for fish fed 4 times/24 h (▽), once/24 h (△), reared in warm water (□) reared in cool water (◇), reared under constant illumination (○), and reared under 12:12 LD (■). Regression calculated for data pooled from all treatments.

12:12 LD regime. This indicates that increment width was greater in fish reared in darkness.

In the photoperiod experiment with 90-d fry, SEM examination of otoliths taken from fish held under constant illumination and the 12:12 LD regime did not reveal any significant differences in the number of increments between the two treatments (t-test, $P > 0.05$). Fish held under constant illumination laid down growth increments whose mean width was significantly less ($P < 0.05$, analysis of variance) than that of fish from the 12:12 LD treatment (1.03 ± 0.16 μm and 1.25 ± 0.25 μm, respectively). However, no significant difference ($P > 0.05$) was found between mean fork lengths of fish held in these photoperiod regimes. Slopes of regressions of otolith size on fish size also did not differ significantly, nor were they different from the slope of the regression representing pooled data from all experimental treatments (analysis of covariance, Fig. 4).

Discussion

Of the environmental variables examined, only feeding frequency affected both increment number and width. Young chinook salmon fed more than once per day produced more than one increment every 24 h, while one feeding per day was

associated with the average production of one increment every 24 h. Our results are in agreement with those of Panella (1980) for *Tilapia* sp. Because several workers have indicated that more than one peak in feeding activity occurs in temperate fish populations (Keast and Welsh 1968; Elliott 1970; McDonald 1973; and others), there is reason to expect formation of more than one increment/24 h in many fish species. However, Marshall and Parker (1982) felt that foraging was not an important factor in otolith increment production as formation began prior to first feeding and continued when fish were starved. The possibility that other environmental cues may act as zeitgebers in the absence of exogenous feeding opportunities was not examined.

The frequency of feeding may not be the ultimate factor determining the number and width of increments. Feeding undoubtedly affects fish activity patterns. The manner in which increment width or number might be affected by activity has not been established although it might relate to activity-induced changes in calcium metabolism.

The linear relationship between fish size and otolith size (Fig. 4) for all experimental conditions studied here indicates that otolith increment width is related to the number of increments deposited. While otolith growth is proportional to fish growth during a given period, the change in otolith size under different feeding frequencies is given by the number of increments deposited multiplied by their average width.

Our results showed that rearing temperature did not influence the number of otolith growth increments deposited, and that increments were deposited daily under constant temperature. Our laboratory results do not support Brothers' (1979) work on temperate stream-dwelling fish which implicated diel temperature fluctuations in otolith increment production. Further, although we found daily increment production at 4°C, Marshall and Parker (1982) showed that increment formation in juvenile sockeye salmon ceased at 5°C.

Water temperature affected fish growth which was in turn reflected in differences in mean increment width. Irie (1960) also described a relationship between temperature and otolith growth and showed that deposition of calcium (the major constituent of the otolith) was directly related to both water temperature and feeding. However, Irie did not assess temperature effects on otolith growth independent of food consumption rates.

Our results from experiments on eggs, alevins, and free-swimming fry suggest that a 12:12 LD photoperiod is not necessary for otolith increment formation. Free-swimming fry reared in constant illumination and alevins incubated in darkness from time of fertilization produced at least one growth increment every 24 h. Campana and Neilson (1982) also found that production of daily increments in otoliths of starry flounders was independent of a diel photoperiod. Taubert and Coble (1977) and Tanaka et al. (1981) found that otolith growth in juvenile fish is primarily controlled by an endogenous rhythm synchronized to photoperiod. In preemergent chinook salmon, however, photoperiod is not likely responsible for the entrainment of daily growth increments. Fertilized chinook eggs may be deposited in 30 cm of gravel, well beyond the vertical extent of light penetration in gravel typically used for spawning (Heard 1964).

We inferred that mean increment width in otoliths of alevins held in darkness was greater than that of alevins held in a 12:12 LD photoperiod as otoliths of alevins reared in darkness were larger. Direct measurements of otolith increment width from free-swimming fry showed that mean width was less in otoliths from fish held under constant illumination than in those held under a 12:12 LD photoperiod. Thus, mean increment width in these experiments can be summarized as follows: constant illumination $<$ 12:12 LD $<$ constant darkness. As the rate of otolith growth is proportional to fish growth, this means that growth was reduced under the 12:12 LD photoperiod and constant illumination. Eisler (1961) also presented results showing greater growth in salmonids held in darkness. His results and those presented here may be explained by the photonegative behavior of salmonid alevins (Dill 1969; Dill 1977). Chinook alevins exposed to the 12:12 LD photoperiod probably expended energy to avoid light, leaving less energy available for growth than in alevins held in total darkness. The photonegative response may continue beyond emergence and yolk sac absorption, albeit in a weakened form (Mason 1976). Hence, the decreased growth (as reflected by increment width) in chinook alevins and fry exposed to light in these experiments may be due to a light-induced increase in activity.

While we found significant differences in otolith increment widths for fry held under 12:12 LD and continuous illumination, no difference in fish fork length was noted. This may be due to comparatively imprecise measurements of preserved fish relative to increment width measurements, and the short duration of the experiment relative to the age of the fish.

Some of the comparability of the experimental results presented here may be reduced as fish of somewhat different ages were used in experiments. For example, for the feeding frequency experiments 90- to 155-d-old fish were used while 45- to 90-d-old fish were used in the temperature experiment. This is probably not a serious problem as the age differences are slight. However, it may be inappropriate to extrapolate our results to other life history stages, as chinook salmon experience several different environmental factors in the estuarine and oceanic environments which could affect increment formation. Furthermore, the utility of otolith microstructure studies for life history stages of temperate fish other than underyearlings is not clear. With the onset of winter, changing environmental conditions including reduced water temperature and prey availability may act to modify the pattern of increment deposition.

In this study, no one environmental variable appeared to be of critical importance in the production of otolith growth increments. The production of one increment/24 h in otoliths of alevins held in total darkness lends credence to the suggestion that an endogenous rhythm is responsible for increment production. Other workers, including Brady (1979), have described a circadian rhythmicity in laboratory-reared animals which had never experienced environmental rhythms of 24-h periodicity. Deviations from the one increment/24 h relationship such as was seen when the chinook salmon received four feedings/24 h may result from an interaction between an endogenous rhythm and some recurring exogenous event such as feeding activity. Considerable latitude probably also exists for modification of increment width through interactive effects of environmental variables, as each

variable examined singly in this paper affected increment width.

Otolith microstructure permits greater resolution of age and growth in young fish than was previously possible using scales or other bony parts of fish, and it offers a means of assessing past environmental conditions that affect growth. However, some recent studies have treated growth increments as daily in their occurrence without corroboration. In a few cases, these growth increments have provided the basis for back calculation of growth. This study shows that while the relationship between otolith size and fish size remains constant under a variety of experimental treatments, both number and width of otolith increments can be modified by environmental variables. Therefore, the derivation of back-calculated growth estimates and other inferences based on otolith microstructure should only be undertaken after the frequency of formation of growth increments and the role of environmental variables in their formation is understood.

Acknowledgments

We thank Eldon Stone and the staff of the Capilano Salmon Hatchery for their cooperation throughout this study. Steven Campana, Institute of Animal Resource Ecology, University of British Columbia; Dr M. C. Healey, Canada Department of Fisheries and Oceans; and Dr R. Methot, U.S. National Marine Fisheries Service, provided useful comments on earlier drafts. Barbara Puselja and Mike Bradford helped with otolith preparation. Dr V. Bourne, Department of Biological Sciences, Simon Fraser University, provided valuable technical assistance with scanning electron microscopy. This work was supported by grants from Canada Department of Fisheries and Oceans and the Natural Sciences and Engineering Research Council of Canada.

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Carbon Inputs to Bivalve Mollusks: A Comparison of Two Estuaries[1]

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Corresponding trends in $\delta^{13}C$ values for particulate organic carbon (POC) and the tissues of several filter-feeding bivalve mollusks in the Sheepscot estuary, Maine, indicate assimilation of terrestrial organic matter by these estuarine organisms. An adjacent estuary, the Damariscotta, of similar morphology but receiving little river input shows no such isotope change in either POC or bivalves, but rather a domination by marine isotopic values. Geographically localized or species-specific departures from these trends appear to be related to two mechanisms: (1) the influence of locally abundant, isotopically distinct, aquatic macrophytes in an area of seasonally low flushing; (2) surface-deposit feeding on benthic microalgae with anomolously high $\delta^{13}C$ values.

Key words: $\delta^{13}C$, *Mytilus edulis*, *Mya arenaria*, *Macoma balthica*, POC, estuarine comparisons, bivalve feeding

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Les tendances correspondantes des valeurs de $\delta^{13}C$ du carbone organique particulaire (POC) et dans les tissus de plusieurs mollusques bivalves filtreurs de l'estuaire de la Sheepscot (Maine) indiquent que ces organismes estuariens assimilent de la matière organique terrestre. Un estuaire adjacent, la Damariscotta, de même morphologie mais ne recevant que peu d'eau de la rivière ne montre pas de changement d'isotope de cette nature, tant du POC que dans les bivalves. On y voit au contraire une dominance de valeurs d'isotopes de mer. Les déviations, géographiques ou particulières à l'espèce, de ces tendances semblent liées à deux mécanismes : (1) l'influence de macrophytes aquatiques distincts sous le rapport des isotopes, localement abondants, dans une zone de faible vidange saisonnière ; (2) l'ingestion de dépôts de surface fixés sur des micro-organismes possédant des valeurs exceptionnellement élevées de $\delta^{13}C$.

Received January 4, 1982
Accepted April 15, 1982

Reçu le 4 janvier 1982
Accepté le 15 avril 1982

NUMEROUS studies have examined the flow of organic carbon of different plant sources through estuarine food webs using ratios of the stable isotopes of carbon as natural labels (Haines 1976a, b; Haines and Montague 1979; Thayer et al. 1978; McConnaughey and McRoy 1979a; Hackney and Haines 1980; Fry 1981). This approach has obvious appeal because it presumably enables identification of sources of carbon actually assimilated at the consumer level (DeNiro and Epstein 1978; Fry et al. 1978a, b, c). The various isotopic ratios of plants are apparently related to differences in photosynthetic pathways of carbon fixation (Benedict 1978; Wong and Sackett 1978), and these ratios, expressed as $\delta^{13}C$ values,

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Printed in Canada (J6768)
Imprimé au Canada (J6768)

appear to be only slightly altered by the metabolism of consumers (DeNiro and Epstein 1976, 1978; McConnaughey and McRoy 1979b). However, for various aquatic plants, the distinction between photosynthetic pathways (Beardall et al. 1976) and relationship to isotopic ratios (Benedict et al. 1980; Morris 1980) appear to be less straightforward than once thought. In addition, the range of $\delta^{13}C$ values for primary producers of interest may be narrow and the trophic relationships of consumer populations complex. Consequently, the $\delta^{13}C$ values of many estuarine consumers fall into a narrow range where implied pathways of carbon flow are difficult to demonstrate with any confidence (Fry and Parker 1979).

One method for evaluating the significance of relatively small differences in $\delta^{13}C$ of a consumer species, and thereby supporting the conclusion of carbon input from a particular source, is to look for systematic trends in specimens along a fairly lengthy gradient of differing source potential. A gradient of progressive mixing of terrestrial and marine inputs to an estuarine food web represents such a situation. We report here organic carbon $\delta^{13}C$ values of suspended particulates (seston), surficial sediments, potential sources of detrital carbon, and the tissues of several bivalve species which occur naturally in longitudinal gradients of sites in two adjacent estuaries in the northeastern United States. To our knowledge, this study is unique in examining biota at frequent intervals over estuarine gradients of this length, where systematic trends can become obvious.

Materials and Methods

This study was conducted in two adjacent estuaries along the central coast of Maine, USA (Fig. 1). Although alike in other respects, these estuaries differ markedly in their land drainage areas and freshwater discharge levels and thus offer potentially different mixtures of terrestrial and aquatic production contributing to total carbon flow. The Sheepscot River drains an area approximately four times that drained by the Damariscotta and remains appreciably diluted by freshwater throughout the year. In addition, there are connecting waterways to a major river system to the west (the Kennebec River) at ~ 8 and 25 km from the Sheepscot mouth. The Damariscotta watershed is restricted to a small area surrounding the estuary. Most freshwater flow derives from an impounded lake at the head of the estuary, with relatively little freshwater input during summer months. Similar tidal amplitudes, climate, geomorphic features, and ranges of water temperature are found in both estuaries.

Sampling sites ranged from the mouth of both estuaries to 30 km upestuary in the Damariscotta River and 33 km in the Sheepscot. In both estuaries, the uppermost sites were near the head of tidal influence.

The bivalves examined in this study include epifaunal filter feeders (*Mytilus edulis*, *Modiolus modiolus*, *Geukensia demissa*), infaunal suspension feeders (*Mya arenaria*, *Mercenaria mercenaria*), and an infaunal surface-deposit feeder (*Macoma balthica*). Specimens were collected in spring, summer, and autumn and placed in filtered seawater for 24–48 h to allow depuration of most of the gut contents. Soft tissues were removed from the shell, the gut region excised in most cases, and the remaining tissues placed in 1 mol/L HCl

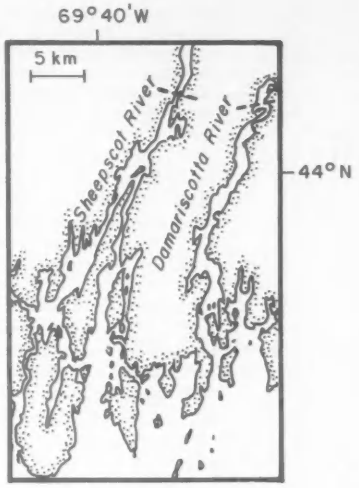

FIG. 1. Sheepscot and Damariscotta River study areas along the coast of Maine. Broken lines indicate uppermost sites sampled in each estuary.

for 10–15 h to dissolve carbonate material accidentally chipped from the shell or contained in pearls embedded in the mantle tissue. Tissues were subsequently dried at 60°C for 48 h and ground to a powder for analysis. In the few cases where the gut region had not been removed, comparison with locally collected specimens which had received this treatment were not significant. Surficial sediments were collected by scraping the top 0.5 cm with a laboratory spatula. Intertidal sediments were collected at low tide; subtidal samples were obtained with a Shipek grab. (Subtidal sediments are the same as in Mayer et al. (1981).) Seston samples were collected by filtering water samples through several precombusted Gelman type A/E filters. Benthic filamentous algae, marsh grasses, and forest litter from estuarine channels and forest floor were rinsed, acid-treated, dried, and powdered. Combustion methods followed Haines and Montague (1979) and results are reported as $\delta^{13}C$ relative to PDB. Standard deviation of analysis was 0.5‰ or less.

Transplant experiments were conducted with experimentally rafted mussels, *M. edulis*, taken from rafts in the middle portion of the Damariscotta and transferred to identical rafts in the upper Sheepscot estuary. Mussels which had set naturally on the rafts in both locations were retained as controls. The experimental mussels were transplanted in May and collected in November.

Results and Discussion

The $\delta^{13}C$ values of particulate organic carbon (POC) and bivalve tissues for the two estuaries are listed in Table 1, along with the distance of the collecting site from the estuary mouth. POC samples showed little isotopic change over the length of the Damariscotta River sampled, but in the Sheepscot a systematic landward decrease of $\delta^{13}C$ was found. This

TABLE 1. Estuarine trends in $\delta^{13}C$ values for particulate organic carbon (POC) and bivalve tissues.

Distance from estuary mouth (km)[a]	POC $\delta^{13}C$ values (‰)[b]	Bivalve tissue $\delta^{13}C$ values (‰)	Bivalve species[c]
		Damariscotta River	
−6.8	−21.2		
−3.4	−21.0		
−0.9	−21.7		
0	−22.0		
2.2	−21.6	−18.2	1
11.3		−18.4	2
12.2	−21.4		
13.3	−21.7	−18.8, −18.4, −18.3, −17.9[d]	1
		−18.2, −17.7, −17.6	2
		−17.7, −16.6[d]	3
25.9		−17.3	2
		−16.6	3
30.3		−17.0	3
		−16.5	5
		Sheepscot River	
0		−18.7	1
1.4	−23.8		
4.8		−19.3	1
		−19.4	4
6.9	−23.9[e]		
8.1		−18.4	2
10.5		−19.3	1
12.2		−19.4	1
13.5		−19.6	1
14.4	−24.0		
17.8	−24.1		
23.8	−24.0	−19.2	2
		−20.1	1
24.7	−24.6		
27.3	−24.6	−21.1	2
		−21.8	1
28.8	−24.6		
32.2		−21.4	1
		−21.8	2
32.8	−25.0		
		Sheepscot River Tributaries	
30	−24.2	−22.5	1
34.5	−25.0	−22.0, −22.5	3
	−25.3[e]		

[a]Negative values indicate distance seaward (offshore); others indicate distance up-estuary.

[b]Surficial sediment values reported here except as noted in footnote e.

[c]Species are: 1, *Mytilus edulis*; 2, *Mya arenaria*; 3, *Geukensia demissa*; 4, *Modiolus modiolus*; 5, *Mercenaria mercenaria*.

[d]Multiple values are from specimens taken from a variety of substrates and habitats within the area.

[e]Seston values.

latter trend is consistent with an increase in the relative contribution of isotopically light, terrigenous detritus to the POC pool (leaf litter, soils, and sediments from the surrounding hardwood forests and in stream and river channels had $\delta^{13}C$ values from −25‰ to −28‰). Whereas other explanations are certainly possible (see following paragraphs), the view that this trend in $\delta^{13}C$ values is due to increased terrigenous input at progressively upriver sites was corroborated by independent investigations of the source of organic matter to the surface sediments by Mayer et al. (1981), who also confirmed a relatively uniform dominance of marine carbon in the Damariscotta POC samples.

The isotopic composition of tissues from bivalves collected along longitudinal transects in both estuaries systematically followed the trends observed for POC. The tissues of bivalves collected at progressively landward sites in the Sheepscot

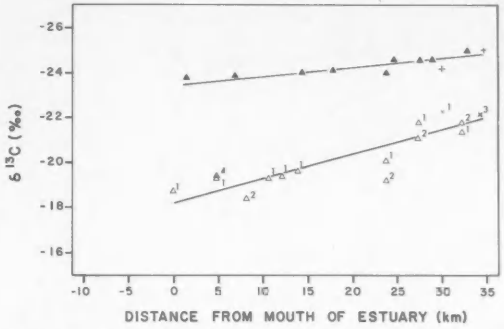

FIG. 2. $\delta^{13}C$ values and least squares regression lines for particulate organic carbon (POC; ▲, $r^2 = 0.77$) and bivalve tissues (△, $r^2 = 0.73$) at varying distances from the mouth of the Sheepscot estuary: (1) *Mytilus edulis*; (2) *Mya arenaria*; (3) *Geukensia demissa*; (4) *Modiolus modiolus*. Slopes of both regression equations are significantly different from zero ($P < 0.01$) and from each other ($P < 0.05$) (Student's *t*-test). POC (+) and bivalve (x) $\delta^{13}C$ values for two tidal tributaries to the Sheepscot are plotted. Table 1 provides a comparison with Damariscotta data.

estuary showed progressively lighter isotopic composition: the $\delta^{13}C$ values of *M. edulis* ranged from −18.7‰ to −22.5‰, and those of *M. arenaria* from −18.4‰ to −21.8‰, from the mouth to the upper portion of this estuary. Other species sampled in this estuary showed values very close to those of *M. edulis* and *M. arenaria* when collected from the same areas (Fig. 2). The tissues of *M. edulis* and *M. arenaria* from the lower 13 km of the Damariscotta River showed no increase in $\delta^{13}C$ landward, but rather showed relatively uniform values corresponding to the marine-dominated $\delta^{13}C$ values of POC. At the most seaward sites in both estuaries, bivalve tissue $\delta^{13}C$ values were similar and had typical marine values taking into account some enrichment in ^{13}C due to animal metabolism (DeNiro and Epstein 1978).

The results of the transfer experiment (Table 2) substantiated both the interestuarine comparison and the Sheepscot estuary trend. The $\delta^{13}C$ values of mussels transplanted from the Damariscotta to the Sheepscot changed from −18.3‰ to −21.2‰, which clearly suggests that the very gradual but consistent trend over a somewhat greater range of values from the mouth to the upper estuary of the Sheepscot is, indeed, a trophically significant one. Because the POC $\delta^{13}C$ trend in the Sheepscot appears to result from terrigenous input, the corresponding bivalve trend suggests that a measurable terrestrial influence on the organic carbon composition of bivalves exists as well, though the form of the terrestrially derived carbon used, or its mechanism of incorporation into the estuarine food web, cannot be discerned from our data. We do not consider it likely that dissolved inorganic carbon (DIC) from the Sheepscot River substantially affects estuarine phytoplankton $\delta^{13}C$ because of the rapid dilution of riverine DIC by seawater DIC in this system (unpublished data). The pathway of terrigenous carbon flow into this component of the estuarine food web would thus appear to be organic. These results corroborate the suggestion of Hackney and Haines (1980) that

TABLE 2. Changes in $\delta^{13}C$ values of mussels (*Mytilus edulis*) transplanted from the middle Damariscotta estuary to the upper Sheepscot estuary, compared to those retained in the Damariscotta (single observations for controls; mean ± 1 SD for experimentals, $n = 3$).

Damariscotta River	
Controls	−17.87‰
Experimental	−18.37±0.04‰
Sheepscot River	
Controls	−22.52‰
Experimental (transplants from Damariscotta estuary)	−21.16±0.05‰

terrestrial organic matter can be incorporated into estuarine food chains.

It is conceivable, as an alternative to the above explanation, that the Sheepscot bivalve trend results from a gradient of algal $\delta^{13}C$ values. This could occur if isotopically distinct microalgae found in abundance in the upper Sheepscot were progressively replaced by algae with typically marine isotopic composition seaward. According to current knowledge of pathways of carbon fixation and metabolism in phytoplankton (Morris 1981), differences in algal types and physiological condition of algal populations could conceivably produce such an effect. This mechanism would imply, however, that little of the non-phytoplankton material in the seston is utilized by the filter feeders examined in this study, and there is no published evidence to suggest that such selection (either in feeding or in assimilation) takes place at the level of efficiency required for this explanation to hold true.

Although bivalves from the mouths of both estuaries had typically marine $\delta^{13}C$ values, POC samples from the mouth of the Sheepscot clearly did not. In fact, the Sheepscot POC values from the entire lower half of the estuary undergo a smaller change in $\delta^{13}C$ than would be anticipated from the bivalve data. We interpret that this occurs because only the more refractory components of terrestrial organic matter remain by the time this material is delivered to the lower estuary. The discrepancy between the slopes of the POC regression and the bivalve regression (Fig. 2) may thus be explained by the fact that the sedimentary organic matter derives part of its carbon isotope composition from the more refractory component of organic material delivered to it, while bivalve tissues tend to be made from a more labile component. This labile component must be primarily of marine origin at the mouth of this estuary. The large influence of terrestrial material on surface sediments of the lower Sheepscot is probably due in part to the large riverine flows of the Kennebec River, which interconnects with the lower Sheepscot estuary. Without this interconnection, the discrepancy between $\delta^{13}C$ values of POC and bivalves might not be as great.

Whereas the general pattern of Damariscotta POC and bivalve $\delta^{13}C$ values is consistent with a system dominated by marine primary production, two departures from these typically marine numbers are noteworthy. In the upper estuary, there is an increase (opposite the Sheepscot trend) in $\delta^{13}C$ values of three bivalve species (Table 1), including two species represented in samples collected lower in the estuary. This may result from the input of carbon derived from exten-

sive local beds of the aquatic macrophyte *Zostera marina*; this area of the estuary has low flushing rates for much of the year. McConnaughey and McRoy (1979a) found a similar enrichment of ^{13}C in *Mya arenaria* collected from an environment of extensive *Zostera marina* beds. Again, the mechanism of influence on the isotopic composition of the bivalves cannot be discerned from our data but is of particular interest because of its apparently localized impact.

A second departure from the typically marine values observed for bivalves over most of the Damariscotta was the value for a pooled sample of several specimens of the surface deposit feeding bivalve *Macoma balthica* collected from an intertidal mud flat in the middle portion of this estuary. These samples yielded $\delta^{13}C = -9.7‰$, considerably more enriched in ^{13}C than other, sympatric bivalves or values reported for this genus in the literature (McConnaughey and McRoy 1979a). Bulk surface sediments from the surrounding mud flat had $\delta^{13}C$ values in the range $-19‰$ to $-22‰$. However, filamentous benthic algae (*Amphipleura rutilans*) collected on the surrounding flat had $\delta^{13}C = -12.4‰$, a value suggestive of C_4 carbon pathways (Beardall et al. 1976; Morris 1980). Our data indicate that *M. balthica* is assimilating with high efficiency, or selectively feeding upon (Wernstedt 1942; Hylleberg and Gallucci 1975), a component of the carbon pool which is diluted by isotopically lighter carbon when bulk surface sediments are sampled. Only large, epibenthic *A. rutilans* chains were sampled, but free-living benthic diatoms may yield similar isotope ratios (Morris 1981) and may result in the isotopically heavy values observed for *M. balthica* in our study. The distinctive isotopic composition of *M. balthica* indicates a minor role for suspension feeding in this species in this environment, at least for a substantial portion of the year (cf. Braefield and Newell 1961).

Acknowledgments

We are grateful to J. I. Hedges, R. A. Lutz, and J. E. Richey for many helpful comments; L. Knowles, R. Ruppersberg, and D. Carlson for help with the laboratory analyses; and B. J. McAlice for diatom identification. This research was supported by NSF grant DEB-77-20359 and NOAA Sea Grants 04-7-158-44034 and R/LRF-45.

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Spawning Behavior and Early Life History of Pink Salmon (*Oncorhynchus gorbuscha*) in the Great Lakes[1]

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KWAIN, W. 1982. Spawning behavior and early life history of pink salmon (*Oncorhynchus gorbuscha*) in the Great Lakes. Can. J. Fish. Aquat. Sci. 39: 1353 – 1360.

Pink salmon (*Oncorhynchus gorbuscha*) from a single introduction to Lake Superior in 1956 have reproduced naturally and expanded into all the Great Lakes. Pink salmon feed at or near the surface as they approach spawning streams and some can still be found with food in the stomach on the spawning bed. Spawning behavior of these freshwater pink salmon is similar to that of marine stocks. Fecundity was 1060 ± 229 (24) per fish and total weight averaged 0.68 kg. Total mortality of embryos to 50% hatch ranged from 67 to 87% at five test temperatures (7, 9, 11, 13, 15°C); 15°C is considered to be the upper limit for freshwater pink salmon incubation. Juveniles moved downstream only at night and all juveniles usually took a month to complete this movement. Mean preferred temperature for pink salmon up to 36 wk old was 10°C. Substantial numbers of pink salmon progeny of the 1975 year-class, which entered the Michipicoten River on Lake Superior to spawn in 1977 survived to, and matured at, each age from II to IV yr in a freshwater laboratory environment.

Key words: pink salmon, feeding, spawning behavior, fecundity, incubation, emigration, preferred temperature, early life history, age IV, late maturation

KWAIN, W. 1982. Spawning behavior and early life history of pink salmon (*Oncorhynchus gorbuscha*) in the Great Lakes. Can. J. Fish. Aquat. Sci. 39: 1353 – 1360.

Les saumons roses (*Oncorhynchus gorbuscha*) d'un seul peuplement introduit dans le lac Supérieur en 1956 se sont reproduits naturellement et répandus dans tous les Grands Lacs. Ces poissons se nourrissent à la surface ou près de la surface au moment où ils atteignent les cours d'eau de ponte, et on en trouve sur les frayères qui ont encore de la nourriture dans l'estomac. Le comportement reproducteur de ces saumons roses confinés en eau douce est semblable à celui des stocks marins. La fécondité est de 1 060 ± 220 oeufs (24) par poisson et leur poids total moyen de 0,68 kg. La mortalité d'embryons à 50 % d'éclosion est comprise entre 67 et 87 % à cinq températures d'essai (7, 9, 11, 13, 15°C); on considère 15°C comme limite supérieure de température d'incubation des saumons roses d'eau douce. Les jeunes ne descendent le cours d'eau que la nuit, en une migration qui se termine pour tous les saumons en un mois environ. La température préférée moyenne des saumons roses jusqu'à l'âge de 36 sem est de 10°C. Un nombre substantiel de descendants de saumons roses de la classe d'âge de 1975, qui avaient pénétré dans la rivière Michipicoten du lac Supérieur pour y frayer en 1977, survécurent et atteignirent la maturité à chacun des âges II à IV dans un environnement d'eau douce en laboratoire.

Received February 2, 1982
Accepted June 21, 1982

Reçu le 2 février 1982
Accepté le 21 juin 1982

PINK salmon (*Oncorhynchus gorbuscha*) originating from a single introduction of fry to Lake Superior in 1956 have reproduced naturally for the past 24 yr, and have recently expanded into all the Great Lakes (Lake Huron by 1969; Lake Michigan by 1973; Lakes Erie and Ontario by 1979). These pink salmon form the only known self-perpetuating population in freshwater, and their increase and spread has been remarkable (Collins 1975; Kwain 1978; Kwain and Chappel 1978; Wagner and Stauffer 1980; Kwain and Lawrie 1981; Emery 1981). They are now regarded as one of the established exotics in the Great Lakes.

The objective of this study was to contribute an understanding of the life history of this new element in the Great Lakes fish fauna by investigating feeding behavior of spawners at the time of stream entry, size of spawning run and spawning behavior, fecundity, egg incubation, downstream migration of alevins, preferred temperature for juvenile pink salmon, and late maturation.

[1]Contribution No. 82-03 of the Research Section, Fisheries Branch, Ontario Ministry of Natural Resources, P.O. Box 50, Maple, Ont. L0J 1E0.

Printed in Canada (J6802)
Imprimé au Canada (J6802)

Materials and Methods

Feeding Behavior of Spawning Populations

In August/September 1979, 635 stomachs were collected from maturing pink salmon during their spawning migration into the Steel River (Fig. 1). Details of the location and methods of collection can be found in Kwain and Lawrie (1981). The stomachs were sorted according to the location from which they were taken (in the lake approaches, in the river mouth, in the river proper, and on the actual spawning grounds). Stomachs containing food items were preserved in 10% formalin and their contents subsequently enumerated in the manner of Windell (1968).

Estimates of spawning populations were first made in 1977 in the Michipicoten River and again in the Steel River in 1979. For both rivers, runs were estimated to be ~ 90 000 fish by methods described by Kwain and Lawrie (1981). In 1979, estimates were also made of the numbers actually present on the Steel River spawning grounds on three separate occasions during the run by similar mark/recapture methods.

Fecundity, Egg Incubation, and Fry Emergence

Fecundity of female pink salmon was determined for a sample of those caught in the Michipicoten River in 1977. Weights of the 24 individual fish were determined in the fresh condition as was the weight of 100 eggs from a randomly chosen female. Egg numbers for each fish were subsequently determined by counting all the eggs in each ovary after preservation.

Egg incubation and fry emergence were studied in 1974, 1977, and 1979, both in the field and the laboratory. Recently collected fertilized eggs (after being waterhardened for an hour) were shipped immediately to the laboratory and placed in constant water temperature tanks (7, 9, 11, 13, and 15°C) for incubation. Rearing procedures were identical with those described for rainbow trout (*Salmo gairdneri*) by Kwain (1975). Light intensity in the rearing room was held at about 200 lx at the water surface during working hours, 08:00 to 17:00. Photoperiod was determined by natural daylight via windows and supplemented by the fluorescent lights during working hours. Water temperatures were maintained by thermal regulators and monitored by a Dickson minicoder. Mean hatch time was calculated from the day on which 50% of the embryos had hatched. Mortality, development, water temperature, and oxygen levels were recorded daily.

In the fall of 1979, 10 wooden-frame boxes, each measuring 46 × 46 cm, 30 cm deep, and covered with nylon mosquito screen, were filled with 1−3 cm gravel. Each contained about 1000 newly fertilized eggs from a single pair of parents. Each box was completely sealed to prevent any escape of eggs or alevins or intrusion by predators. The boxes were buried in the bed of the Steel River to determine the embryonic development rate under natural conditions. No thermograph was installed due to the severe winter conditions.

Fry Emigration

In 1980, downstream migration was monitored in the Pancake and Carp rivers, just north of Sault Ste. Marie, by suspending four plankton nets (30-cm diameter) in a row across the river near the mouth during April and May. The nets were checked daily except for weekends. Soon after completion of the downstream emigration from the Carp and Pancake rivers, considerable effort was devoted to searching for the fry in the lake by trawling, beach seining, and scuba diving in the vicinity of the river mouths and nearshore areas.

To determine when the newly hatched fry start to move downsteam, an artificial stream was simulated in the laboratory. A 120 × 240 cm plywood box was divided into eight 15-cm-wide channels connecting at alternate ends to allow continuous flow through each channel in turn. Each end was equipped with a trap where fish could only move in, but not out. Water velocity was adjusted according to the swimming capability of the fry, so that the opportunity for them to move upstream was not inhibited. The velocity ranged between 3.5 and 4.5 cm/s, and water temperature was maintained at 5−6°C. Fish to be tested were first caged in the middle of the stream, then after a ½-h acclimation time, the cage was lifted. Fish could then move freely in either direction. To evaluate migration, the traps were checked each day and after the counting, the fish were removed and replaced by a new group.

Preferred Temperature

The thermal preference of pink salmon (ages 23−36 wk) was studied in the research laboratory using a vertical thermal gradient tank (Pearson 1952), a horizontal linear gradient tank (Sullivan and Fisher 1953), and a shuttlebox (Neill et al. 1972). Procedures and methods were those described by Kwain and McCauley (1978). Test fish were of Lake Superior origin, incubated and reared in the research laboratory. Prior to testing, they were acclimated to 10 ± 0.5°C. All experiments were conducted in a light-proof room. For each determination, 20 fish were chosen at random from the acclimation tank and immediately transferred to a preferendum tank when a gradient had partly developed. They were left in the tank for 30 min in total darkness to habituate. When the temperature gradients had been established, a 15-W incandescent bulb suspended about 60 cm above the tank was turned on and provided light intensities of 220−222 lx from the surface to the bottom of the tank. The first observation was made 10 min later. Positions of fish were recorded both photographically and visually. Observations were made at 10-min intervals over a period of 100 min. Temperature gradients were recorded at the time of each observation.

All determinations of temperature selection were carried out in the morning, and at the conclusion of each experiment, the heating and cooling coils were shut off and isothermal conditions achieved by mixing the water through aeration. Control experiments were conducted on the afternoon of the same day with the tank maintained at the acclimation temperature of the sample.

The vertical gradient tank consisted of a glass aquarium (125 cm deep, 92 cm deep, and 61 cm wide). One of the broad sides of the aquarium was designated as the front and horizontal lines were drawn on the glass to delineate 10 equally sized zones (temperatures ranged 6 to 20°C). Temperatures at the midpoint of each observational zone were read from a

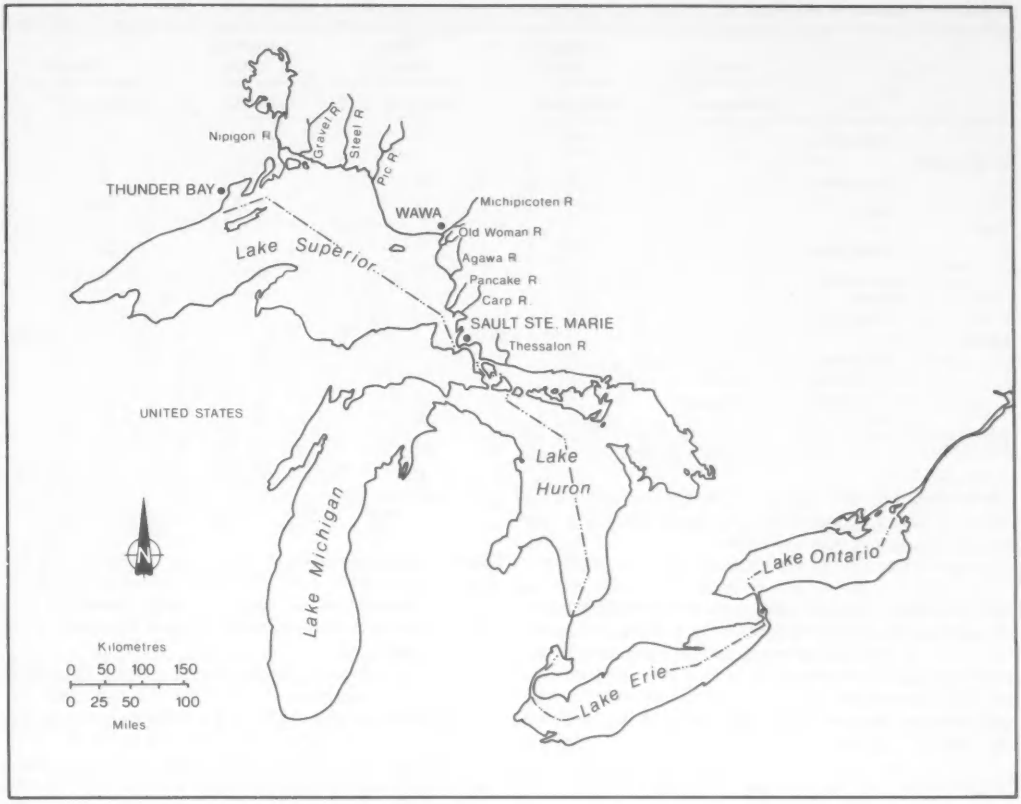

FIG. 1. Pink salmon study areas, tributaries on the north shore of Lakes Superior and Huron.

12-channel telethermometer via 10 YSI (Yellow Spring Institute) interchangeable thermistor probes.

The horizontal gradient tank consisted of a trough constructed of glass (30 × 30 cm and 180 cm long). The tank was divided into 10 observational zones of equal width by transverse lines. Water, cooled in a reservoir to 4−6°C, was introduced into zone No. 1 through a plastic 'tee' leaving the opposite end through a standpipe. The standpipe also served to maintain the depth of the water in the trough at 10 cm. Horizontal temperature gradients were established by progressively heating the water as it flowed the length of the trough with 2000-W electric heating coils which were imbedded into a layer of fine gravel laid on the bottom of the trough. Desired thermal gradients (spanning 12°C) were achieved by adjusting the flow of the water intake. Vertical stratification of the water was eliminated by 10 air stones which were located at regular intervals along the length of the tank.

Fish were observed through a longitudinal slit in a screen placed in front of the trough which provided a view of a mirror mounted above the tank at a 45° angle and running the length of the trough. This arrangement permitted visual observation of the test fish without disturbing them. Temperatures at the midpoint of each zone were read from a 10-channel YSI telethermometer.

The shuttlebox apparatus used for the experiment was a modification of a similar device developed by Neill et al. (1972). The shuttlebox was constructed entirely of 6-mm Plexiglas and consisted of two truncated "wedge-shaped" chambers (40 × 35 × 35 cm) connected by a short passageway. A pair of photoelectric cells positioned at this passageway monitored the movement of the fish between the two chambers. When the fish occupied the left "warm" chamber, 25°C water was delivered to the system at the rate of 0.8 L/min from a 225-L reservoir. When the fish swam through the passageway to the right "cold" chamber, the photoelectric cells were sequentially interrupted and a control circuit was activated. This shut off the 25°C water supply and turned on a 4°C water supply delivered to the system at the same rate from a similar reservoir. The effluent left the system via a standpipe positioned at the right rear of the cold chamber. Temperatures in the shuttlebox were monitored during the experimental period by a YSI dual channel recorder. The shuttlebox was initially filled with water drawn from the holding tank. One experimental fish was then placed in the appa-

TABLE 1. Frequency of occurrence of individual food organisms in 96 pink salmon stomachs containing some food.

		Santoy Bay		Mouth of Steel R.		Inside Steel R.		Spawning Bed		Overall	
		Occurrence	%	Occurrence	%	Occurrence	%	Occurrence	%	Occurrence	%
Zooplankton	Cladocera	2	23	14	21	0	0	1	17	17	19.8
	Copepoda	1		1		0		0		2	
Fish	Smelt	1	23	12	45	4	67	3	83	20	50.0
	Unidentified	2		20		0		2		24	
Insects	Lepidoptera	3	77	18	58	1	17	0	0	22	54.2
	Diptera	5		12		0		0		17	
	Homoptera	0		2		0		0		2	
	Hemiptera	1		1		0		0		2	
	Orthoptera	0		1		0		0		1	
	Coleoptera	0		1		0		0		1	
	Unknown	1		6		0		0		7	
Mysidacea		0	0	2	3	0	0	0	0	2	
Amphipoda		0	0	1	1	0	0	0	0	2	8.3
Unidentified		1	8	1	1	1	17	0	0	4	
No. of stomachs examined		93		390		28		124		635	
No. of stomachs with food		13		71		6		6		96	

ratus and moved several times back and forth through the passageway by gentle prodding with a glass rod. After ~ 10 min, the apparatus was activated. The average of maximum and minimum temperature at each 15-min period was used as the selected temperature for that period; the total was averaged and the mean was taken as the preferred temperature.

MATURATION

In September 1977, 32 000 pink salmon eggs were stripped from ripe females collected from the Michipicoten River of Lake Superior, fertilized, and incubated in the laboratory. In February 1978, about 900 newly hatched sac fry of the same year-class were taken from Bridgeland Creek, a tributary of the Thessalon River of northern Lake Huron, and added to the laboratory stock. The two lots were reared simultaneously in untreated soft, spring-fed creek water (for chemical characteristics, see Kwain (1975)) until they were mature, and died.

Results

FEEDING BEHAVIOR OF SPAWNERS

Generally, prior to or during spawning, salmon do not feed, but 15% of the maturing pink salmon stomachs contained food. However, these stomachs contained some food items even when the pinks were caught in the river. The percentage of various food items found in the stomachs containing food is tabulated by taxon in Table 1. There was no great difference in content in the four areas, i.e. bay, rivermouth, middle of the river, and spawning beds. However, most stomachs with food from the river proper or the spawning beds contained rainbow smelt (*Osmerus mordax*), which must have been residual from lake feeding because no smelt were found in the river at time.

In the pooled data, fish and insects showed the greatest frequency of occurrence. Zooplankton were next most frequently eaten. Stomachs with fish remains tended to be fullest, probably because smelt remains were digested more slowly. Apparently, pink salmon were feeding at or near the water surface because almost all identifiable insects were terrestrial, especially the moths and flies. There was little evidence that pink salmon fed on benthic organisms.

SIZE OF SPAWNING RUN AND SPAWNING BEHAVIOR

The estimated number of fish on the major Steel River spawning bed in 1979, at three different times, is shown in Fig. 2. The spawners present each time were considered to be exclusive of earlier or later groups, because no fish marked earlier were found in the next collection of fish examined. This meant that fish present during each collection had completed their spawning activities and moved out of the area and/or died before the next collection was made. Thus, the spawning cohorts remained on the spawning areas for less than 1 wk before replacement.

The physical characteristics of the pink salmon spawning beds in the Steel River are on record (Kwain and Lawrie 1981). The spawning behavior of the salmon was similar to that described for Pacific coast anadromous stocks (Scott and Crossman 1973). The female digs a redd and is attended by several males. After shedding her eggs, she digs gravel from upstream to cover the fertilized eggs. The female does not honor redds made by other females. At a preferred spawning area, redd sites were used many times by different females.

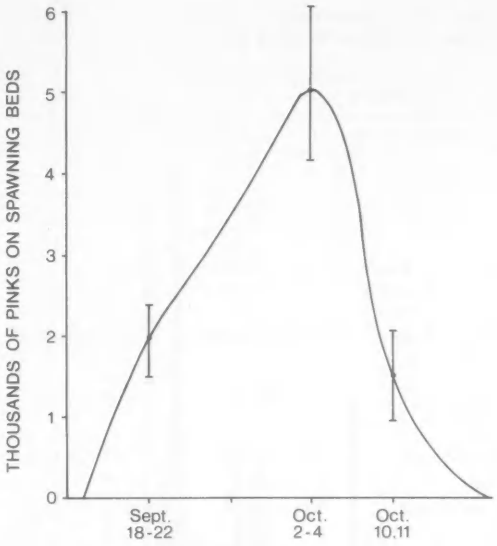

FIG. 2. Estimates of pink salmon population size on spawning beds at different time periods in Steel River, 1979. (Vertical bars represent 95% confidence limit.)

Previously laid eggs were dug up and drifted downstream. Most such dislodged eggs would die.

FECUNDITY, EGG INCUBATION, AND FRY EMERGENCE

Fecundity of females caught in the Michipicoten River in 1977 is shown in Fig. 3, together with some available data from the Pacific coast (Bams 1973; IPSC 1979). The average number (± SD) of eggs per each female was 1060 ± 229 (24 samples). Average weight of the females was 0.68 kg. The regression of egg number on weight of fish was calculated as:

$$Y = 616 + 656X.$$

The average diameter of eggs was 6.38 ± 0.28 mm and their weight was 0.118 ± 0.012 g (100 samples). Total length of newly emerged fry from the Steel River in 1976 averaged 30.1 ± 0.9 mm (23 samples).

Total mortality of pink salmon embryos to 50% hatch ranged from 67 to 87% at five constant temperatures (7, 9, 11, 13, 15°C). The results are summarized in Table 2. During the incubation period, the high mortality rate was always observed at two periods; one prior to the eye formation and the other just before hatching. Of the eggs incubated at 15°C, all were dead 166 d after fertilization and the larvae were tiny, sluggish, inactive in feeding, and clearly of inferior quality before death. Therefore, 15°C is considered to be the upper limit for Great Lakes pink salmon egg incubation.

In the spring of 1976, spawning redds in the Steel River were dug to assess embryo development under natural conditions. The incubation period for embryos under ice was estimated from the time of peak egg deposition to the stage of development at the time of digging. The eggs survived the near-zero winter water and took about 170 d to 50% hatch.

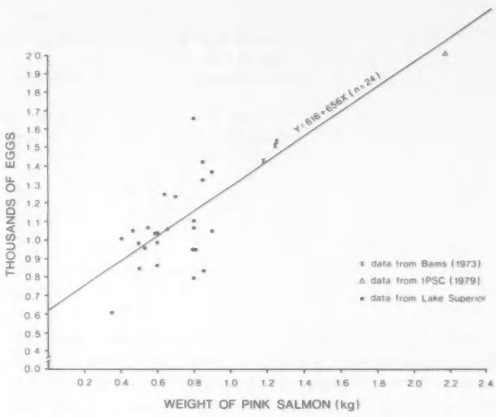

FIG. 3. The relationship between number of eggs and female pink salmon size, Michipicoten River, 1977.

In 1979, 10 boxes, each containing about 1000 eggs, stripped from a single female, were buried in the stream bed. In 1980, only two of them were recovered undamaged; Box 1 on February 6, Box 2 on April 9, whereas a third on April 10 was damaged but still contained some eggs and alevins. All fry were alive and the results are as follows:

Box No.	Eggs dead	Eggs alive	Fry with yolk sac	Fry without yolk sac
1	139	168	32	12
2	84	—	25	26
3	64	—	8	—

Of the eggs that had hatched, considerable differences in the stage of development were apparent even in the same lot. High mortality occurred despite the protection from predation afforded by the boxes. On the basis of the disappearance of dead embryos inside the boxes, a high rate of putrefaction of dead embryos apparently occurred.

FRY EMIGRATION

In the artificial stream, the percentage of fry trapped in a downstream trap was positively related to their age. Three weeks after emerging from the bottom, 50% of the fry moved downstream. This percentage increased as they grew older and at age 8 wk, all completed their emigration. The results were summarized on a weekly basis as follows:

Age (wk)	1	2	3	4	5	6	7	8
% downstream	12	42	52	65	55	87	95	100

It was found also that the fry moved downstream only at night, even when the artificial stream was covered by a dark plastic sheet by day.

TABLE 2. Mortalities (%) and incubation periods of pink salmon embryos from Lake Superior exposed to five constant water temperatures. (Six replicates for each temperature, only five for 15°C.)

Water Temp. °C	No. of eggs	Eyed-up, % dead	50%hatch		166 d, % survival
			Days	% dead	
7	2191	49	74	67	1
9	2878	73	69	74	12
11	2321	51	62	68	19
13	2613	66	51	87	1
15	2167	56	47	74	0

In Pancake and Carp rivers, about 75 km north of Sault Ste. Marie, pink salmon fry started to move downstream in the 1st week of May. The run peaked early in the month and emigration was complete at the end of the month (Fig. 4). The number appearing in the Pancake River was much greater than in the Carp River, coinciding with the relative sizes operated on the weekends. On May 12, 1980, the nets were checked at $\frac{1}{2}$-h intervals from 10:00 to 16:00. No fry were caught at this time, but fry were caught during the overnight setting, thus confirming the laboratory observation. There was no significant size difference between early and late season migrants. The numbers of fry emigrating during May was calculated by extrapolation of the net catches on the basis of the river cross section fished by the nets. The estimates for the Pancake and Carp rivers were 60 000 and 19 000, respectively.

After completion of downstream migration, an extensive search for young pink salmon was conducted at river mouths and nearshore areas; however, no pink salmon were caught.

PREFERRED TEMPERATURE

The mean preferred temperature of pink salmon fry was 10.3°C. There were no significant differences ($F = 0.26$, $P > 0.25$) among results from the three different apparatuses used in testing (Fig. 5). Also, no significant difference was found at various ages of pink salmon tested or three different apparatuses ($F = 0.58$, $P > 0.25$).

LATE MATURATION

In September 1979, 76% of the survivors from two lots (Michipicoten and Thessalon rivers, 1977 year-class), matured, were spawned, and died. During maturation, coloration darkened on their backs, large black dots appeared on both lobes of the tails, and the silvery sheen on the bellies diminished. Males did not develop the characteristic pink salmon hump on their backs, perhaps, because of their small size. The remaining fish did not mature and retained their bright green backs and silvery sides. Among those that matured, males outnumbered females 2:1 (Table 3). Among those that did not, all were females except one. Some of these survived to, and matured in 1980, as 3-yr-olds, others in 1981 as 4-yr-olds.

The mean lengths and weights ± SD (N) of the fish maturing in each of these 3 yr are as follows:

Year and age	Fork length (mm)	Total weight (g)
1979		
Two-yr-old	231±37 (51)	108±62 (51)
1980		
Three-yr-old	265±19 (35)	162±38 (38)
1981		
Four-yr-old	302±30 (43)	215±97 (43)

In contrast, pink salmon of the 1977 year-class taken from Lake Superior spawning runs in 1979 averaged 358 mm in fork length for both sexes, whereas data from a pooled Lake Huron/Lake Michigan sample averaged 389 mm for both sexes. Though these laboratory-reared fish were small in size (51−274 g), they did produce viable eggs and the second generation still lives in the laboratory where they are exhibiting the same slow growth as their parents.

Discussion

Pink salmon are the most-abundant salmon in the eastern Pacific. Average production from this fishery over 50 years (1919−70) was about 20 000 t (Hart 1973) and accounted for about 52% of the commercial salmon harvest on the Canadian west coast. In the Great Lakes environment the species has not only established the world's only known, landlocked, self-perpetuating population, but expanded and dispersed with rapidity which suggests that it can be expected to establish major populations throughout the lakes. It is already of some importance to the commercial and angling fisheries (Kwain and Lawrie 1981) and is likely to become more so. It must also be reckoned to be a major new element in the fish community with unknown implications for its integrity and stability. In these circumstances, it is not only important to establish the major features of the life history of the landlocked form but to relate these to the much larger fund of knowledge about its sea-run progenitor.

The spawning behavior of Great Lakes pink salmon is essentially indistinguishable from that recorded for sea-run fish (Hart 1973; Scott and Crossman 1973). Egg sizes do not differ significantly and the eggs are entirely similar in their bright orange−red coloration. Moreover, although Great Lakes pink salmon produce only 1060 eggs per female, as compared to 1500−1900 in the sea-run fish (Hart 1973), fecundity appears to be related to size of fish ($r = 0.54$), the

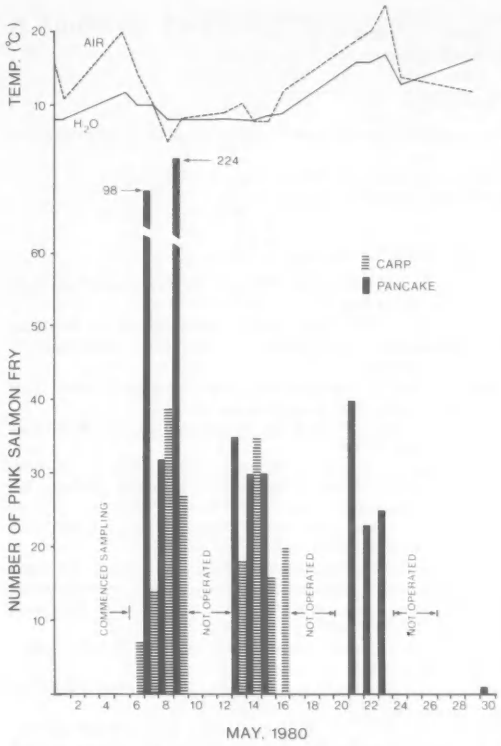

FIG. 4. Number of juvenile pink salmon caught in downstream nets (30-cm diameter) in Pancake and Carp rivers during May, 1980.

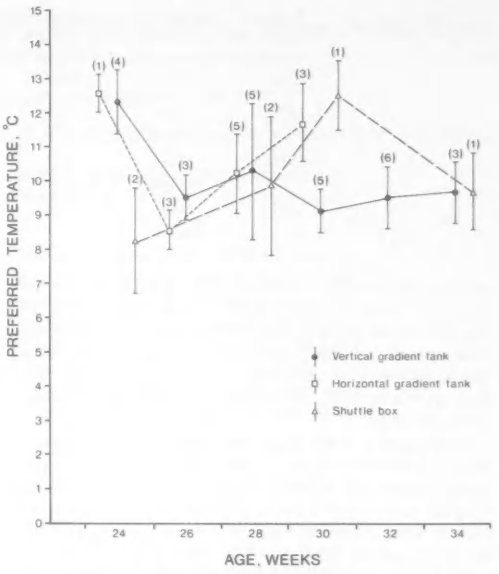

FIG. 5. The mean preferred temperature of juvenile pink salmon in three different apparatus (vertical, horizontal gradients and shuttlebox) at different age-groups (weeks after swim-up). Vertical bars represent standard deviation. Numbers in parentheses represent replicates.

larger the fish, the more fecund as shown in Fig. 2. The spawning behavior, egg diameter, newly hatched fry sizes, and emigration pattern were the same for both; therefore, the landlocked pink salmon appears to be no more than a miniature Pacific pink salmon.

Further, if egg production is likely to be less than Pacific coast expectations would suggest, it also seems likely that mortalities from egg deposition to emergence might be higher. Wydoski and Whitney (1979) reported that development mortalities in Pacific stocks commonly exceed 75%. Very high mortalities were also encountered in our studies, both in the laboratory and the field. Winter conditions on the north shore of Lake Superior are extreme and our field surveys revealed that many redds were completely frozen, at least at the surface of the gravel which had, itself, been obviously scoured. On the other hand, they also showed that many embryos survived these near-zero water temperatures in better locations. These observations are consistent with those of F. C. Withler (personal communication), who has found that embryos of sea-run pink salmon can tolerate near-zero temperatures provided early development has occurred within the preferred temperature range from 7 to 10°C, which would be expected on the Lake Superior north shore immediately following the spawning season. The observation that 15°C appears to be the upper limit for normal development of landlocked pink salmon in our laboratory experiments raises the possibility that the spawners attempting to use lower Great Lake spawning streams may encounter high temperature problems. For example, temperatures on the north shore of Lake Erie streams at the time of the recent spawning runs there are said to have been of the order of 15–17°C (D. Reid, Ontario Ministry of Natural Resources, personal communication).

Pacific coast pink salmon fry generally do not feed in freshwater streams unless the distance to the ocean is great (Wydoski and Whitney 1979; Hart 1973). Similarly the stomachs of emigrant landlocked fry were found to contain no food. Little is yet known of the dispersal of the fry of landlocked pink salmon. Soon after completion of the downstream emigration from the Carp and Pancake rivers, considerable effort was devoted to searching in the lake for the fry. No young salmon were located and the reason for their disappearance is unknown. Hurley and Woodall (1968) reported that the youngest sea-run fry (newly emerged) generally selected temperatures of 11.7–12.8°C in a vertical gradient, whereas older fry (up to 10 wk) were found in temperatures of 9.4–10.6°C. The mean temperature selected by the landlocked pink salmon (from 24 to 34 wk) in our laboratory tests was 10.3°C. The temperature of inshore water in Batchawana Bay was 18–20°C at the time of our survey. The disappearance of fry from the nearshore area might be attributed to their

TABLE 3. Number of two stocks of pink salmon (1977 brood) reared under laboratory conditions that reached sexual maturity at the age of 2, 3, and 4-yr life cycles.

Year	Sex	Age (yr)	Thessalon R.	Michipicoten R.
1979	Male	2	96	230
	Female	2	44	83
1980	Male	3	0	1
	Female	3	3	86
1981	Male	4	0	0
	Female	4	23	22

avoiding unfavorable nearshore water temperatures and seeking cooler water at deeper depths. According to Hart (1973) this move to deep water occurs in September for Pacific coast pink salmon fry. The postulated early movement of landlocked fish to deep water is a difference which might affect their growth adversely as food is generally more abundant in nearshore areas.

In their native, north-Pacific environment, these salmon are obligate anadromes characterized by an essentially invariant, 2-yr life cycle, egg deposition to egg deposition. Three-year-old pink salmon have been reported only twice from the west coast of North America (Anas 1959; Turner and Bilton 1968). In Lake Superior, however, 3-yr-old pink salmon have not been uncommon (Wagner and Stauffer 1980), and their occurrence appears to have allowed establishment of an even-year spawning stock in Lake Superior in addition to the odd-year stock originally introduced (Kwain and Chappel 1978). This phenomenon prompted us to conduct an experiment involving the hatchery rearing of pink salmon.

Pink salmon are slower growing in oligotrophic than their counterparts are in the richer feeding grounds of the north Pacific, and both Wagner and Stauffer (1980) and Kwain and Chappel (1978) have suggested this as the cause of the observed delay in maturity. It is well documented for salmonids (Ricker 1964) as well as for many other groups of fish that on the average, slower-growing individuals in a population reach maturity at an older age and that males generally mature at an average age younger than females. The results of this experiment on pink salmon from Lake Superior are consistent with those biological tenants, even to the surprising extent that some pink salmon (all females) did not mature until age IV.

The plasticity of pink salmon life history in the Great Lakes seems to provide the potential for genetic engineering and the opportunity to establish the long-waiting off-year pink salmon runs in the Northwest.

Acknowledgements

I thank A. H. Lawrie, J. J. Collins, and S. J. Nepszy of the Ontario Ministry of Natural Resources, and F. C. Withler of the B. C. Pacific Biological Station, Department of Fisheries and Oceans, for critical review of the manuscript, and also the staff of the Lake Superior Fisheries Research Unit for their technical assistance.

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A Model for Predicting Pacific Herring (*Clupea harengus pallasi*) Spawn Density from Diver Observations

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SCHWEIGERT, J. F., AND D. FOURNIER. 1982. A model for predicting Pacific herring (*Clupea harengus pallasi*) spawn density from diver observations. Can. J. Fish. Aquat. Sci. 39: 1361 – 1365.

A series of mathematical models were examined to predict the density of Pacific herring (*Clupea harengus pallasi*) spawn in 1-m^2 quadrats from diver observations of the dominant vegetation substrate, the number of layers of eggs on the vegetation, and the density of the vegetation in the quadrat (percent cover). The most appropriate model was chosen statistically and used to make predictions for comparison with egg counts. Outliers were identified and if a plausible biological interpretation was found, these were deleted from the data series. The model provides an efficient and precise means for estimating herring spawn density from diver observations and could be incorporated into a sampling survey design for estimating spawn abundance for stock assessment.

Key words: mathematical model, Pacific herring, *Clupea harengus pallasi*; spawn density, stock assessment

SCHWEIGERT, J. F., AND D. FOURNIER. 1982. A model for predicting Pacific herring (*Clupea harengus pallasi*) spawn density from diver observations. Can. J. Fish. Aquat. Sci. 39: 1361 – 1365.

Une série de modèles mathématiques a été examinée dans le but de prédire la densité du frai de hareng du Pacifique (*Clupea harengus pallasi*) dans des carrés de 1 m^2 à partir d'observations par plongeurs du substrat végétal dominant, du nombre de couches d'oeufs sur la végétation et de la densité de la végétation dans le carré (pourcentage de couverture). Le choix du modèle le plus approprié s'est fait par méthode statistique, et nous l'avons utilisé pour établir des prédictions qui seront ensuite comparées aux dénombrements d'oeufs. Les données périphériques furent identifiées et omises de la série si une interprétation biologique plausible était trouvée. Le modèle est un outil efficace et précis d'estimation de la densité du frai du hareng à partir d'observations par plongeurs. Il pourrait être incorporé dans un programme d'échantillonnage servant à estimer l'abondance du frai en vue de l'évaluation des stocks.

Received September 22, 1981
Accepted June 17, 1982

Reçu le 22 septembre 1981
Accepté le 17 juin 1982

THE management strategy for Pacific herring (*Clupea harengus pallasi*) has evolved from a system of flexible quota controls during the reduction fishery (Taylor 1964) to one aimed at optimizing escapement during the present roe fishery (Hourston and Haegele 1980). The escapement from the fishery is determined by estimating the density (intensity) and extent (total cumulative mileage) of egg deposition on the spawning grounds. By determining the age composition, sex ratio, and fecundity at age it becomes possible to estimate the spawning stock size required to deposit the observed egg numbers (Hourston and Schweigert 1980). Adding this to the total catch provides an estimate of total spawning stock for assessment purposes.

Drastic declines in herring stocks in the late 1960s were not reflected in changes in total length of spawn (Hourston 1980; Humphreys and Haegele 1976). Apparently, changes in either the width or density of spawn were occurring that did not result in changes in the length of spawn on a coastwide basis.

Consequently, a major research effort was initiated in the mid-1970s to refine estimates of egg abundance in major spawnings so that they would permit accurate assessment of stock sizes. Some initial attempts to estimate eggs per square yard on different vegetation substrates (Hourston et al. 1972) were augmented by a sampling program using scuba divers to collect samples of herring spawn from spawnings of varying egg density from a few areas of the coast (Haegele et al. 1979b; Humphreys and Haegele 1976). From this work it became apparent that most of the herring spawn was being deposited subtidally (Haegele et al. 1981) and was consequently being greatly underestimated by shoreline fishery officer surveys (Haegele and Humphreys 1977).

There are two considerations in the process of estimating total egg numbers for a spawning. First, what is the total area of the spawning and second, what is the mean density of eggs

Printed in Canada (J6644)
Imprimé au Canada (J6644)

in that spawning? Ideally, one would like to determine the optimal sampling design with which to survey the total area in a spawning and thereby collect samples of spawn for enumeration. This latter process is tedious, labor-intensive, and expensive. The original program set out to develop a series of keys relating egg density to particular combinations of egg layers, percent cover of plants in a sampling quadrat, and vegetation type (one of six major classes of spawning substrate) which could then be used to calculate the mean egg density for that vegetation type and applied to the relative area of each vegetation substrate in the spawning (Haegele et al. 1979a) to estimate total egg numbers. This would circumvent the necessity of enumerating egg samples for each spawning.

The keys that were developed were based on linear regression equations which calculated egg density as a function of percent cover for several categories of egg layers for each vegetation type (106 parameters were estimated from 1081 samples). In many cases, there were few observations at a particular intensity (number of layers) resulting in inconsistent increases in egg number with number of layers of eggs at a given percent cover.

Although the predictions from the original regressions were subsequently smoothed by a second series of linear regression equations which calculated egg density as a function of layers of eggs for several categories of percent cover (108 new parameters were calculated from the 530 values predicted from the regressions above), we felt that a more rigorous statistical treatment (a model of several parameters) could efficiently explain the data and would result in more precise and hopefully more accurate predictions of herring spawn density.

The objective of this paper is to present a general linear model which can be used to predict herring spawn density from diver observations of layers of eggs, percent cover, and type of dominant vegetation in individual sample quadrats. The problem of incorporating the model into an optimal sampling design to estimate the mean egg density and total spawn deposition in individual spawning localities will be considered in a subsequent report.

Material and Methods

The samples of herring spawn used to develop the model were collected over several years. In 1976 the sampling was concentrated along transects set randomly, perpendicular to shore, and covering the width of the spawning (Haegele and Humphreys 1976). During 1977 and 1978 samples were collected in a number of spawnings of varying intensities to provide observations of egg deposition at unusually high or low percent cover of vegetation and layers of eggs (Haegele and Humphreys 1978a, b). In all cases the samples were obtained by removing all rooted vegetation within a 0.25- or 1-m^2 quadrat. The samples were subsequently separated into fractions by species of vegetation, weighed, and a weighed subfraction sufficient to fill a 1-L jar preserved for egg enumeration. All counts were corrected to eggs/m^2. Samples were grouped according to the dominant vegetation type. At the time of collection the divers also estimated the number of layers of eggs on the vegetation, and the dominant substrate (filamentous or foliose red algae, kelp, rockweed, sea grass, or other brown algae).

The model used to estimate the amount of spawn in a given quadrat taken from an area with vegetation type i has the form:

$$(1A) \quad Y = e^{\alpha} \cdot L^{\beta} \cdot P^{\gamma} \cdot e^{S_i} \cdot e^{\epsilon}$$

or taking logarithms

$$(1B) \quad \log_e Y = \alpha + \beta \log_e L + \gamma \log_e P + S_i + \epsilon$$

where ϵ is a normally distributed random variable with mean 0 and variance σ^2.

In expression (1A), Y is the observed count of eggs in the quadrat, L is the estimated number of layers of spawn, P is the estimated percent of the quadrat covered by vegetation, and the S_i are dummy variables (Draper and Smith 1966) representing the relative effects of different types of vegetation on the observed egg density. The value for the first vegetation type (sea grasses), S_1, is given the fixed value 0. It then follows that for the other five S's, e^{S_2}, ..., e^{S_6} represent the relative amount of spawn associated with that vegetation type compared to vegetation type 1. The parameters α, β, γ, S_2, ..., S_6 are estimated by least squares (Lawson and Hanson 1974).

The log-linear form of the model (1A) was chosen because of the probable multiplicative rather than additive nature of the error processes. For example, the relative error in estimating one layer of eggs is probably much larger than it would be when estimating four or five layers of eggs.

Significance tests were performed to determine whether the parameters differed significantly from their 'natural' values. A natural value for β is 1. This would indicate that when the other factors are held fixed the number of eggs is directly proportional to the estimated number of layers of eggs. In contrast, a value of β lying between zero and one would indicate that the number of eggs does not increase as quickly as the observed number of layers while a value of β greater than one indicates that the number of eggs increased faster than the observed number of layers.

Similarly, a natural value for γ would be 1. This would indicate that the number of eggs are directly proportional to the estimated percentage of the quadrat covered by vegetation. Finally, natural values for the S_i's would be zero. This would indicate that the number of eggs/m^2 is independent of the type of vegetation.

The process used to derive the best model was to include parameters successively in new models and examine the reduction in the residual sum of squares attributable to the additional parameters. The model which provided the most significant fit to the data, as determined by F ratios, was used to predict egg numbers which were compared to the observed values. Predictions of egg numbers were made by use of the parameter estimates from the fitted model and equation 1A:

$$Y = e^{\alpha} \cdot L^{\beta} \cdot P^{\gamma} \cdot e^{S_i} \cdot e^{\epsilon}$$

where the expected number of eggs, $E(Y)$ is given by:

$$E(Y) = e^{\alpha} \cdot L^{\beta} \cdot P^{\gamma} \cdot e^{S_i} \cdot E(e^{\epsilon}).$$

The expected value of ϵ is equal to $e^{\frac{\sigma^2}{2}}$ where σ^2 is the variance of ϵ. We estimated σ^2 as the residual sum of squares divided by the degrees of freedom.

TABLE 1. Parameter estimates for the preliminary model (A) based on observations with one or more layers of eggs and for the final model (B) including all observations. See text for a description of variables.

	Model	No. of observations	Parameter estimates								
			α	β	δ	γ	S_2	S_3	S_4	S_5	S_6
A.	$\log_e \alpha+\beta\log_e L+\gamma\log_e P+S_i$	726	1.767	0.7718		1.025	0.0143	0.0471	−0.2411	0.2641	−0.0768
B.	$\log_e \alpha+\beta\log_e L+\delta\log_e L+\log_e P+S_i$[a]	913	1.915	0.7519	1.003	1.000	0.0216	−0.0269	−0.2704	0.3467	−0.1465

[a]Where β occurs L refers to layers of eggs greater than one while where δ occurs L refers to layers of eggs less than one.

Residuals ($\log_e$ observed − $\log_e$ predicted) were also examined and if they exceeded a value of 3 were treated as outliers and investigated in detail to determine if a plausible biological reason could account for their deviance and hence permit exclusion from the data set. There were a total of 929 observations available from 1976 to 1978. Initially, we felt that the relative error in estimating fewer than one layer of eggs and less than 10% cover would not permit reliable parameter estimates so we excluded them from the data set. In examining residuals for all 929 observations based on a model fit only to the 726 observations at more than one layer of eggs, it was apparent that many of the large residuals were for those observations at less than one layer of eggs. We then included a separate parameter to account for the data structure at less than one layer of eggs and 10% cover to obtain the final predictive model.

Results and Discussion

The model, equation (1B), was fitted initially to the 726 observations with one or more layers of eggs on the vegetation and accounted for ~57% of the total variation. For the sake of brevity, we present only the parameter estimates for the model fitted to the observations with one or more layers of eggs. The parameter estimates for the model suggest that γ is not significantly different from one (Table 1). The parameter estimate for β is 0.7718 and indicates that the estimated layers of eggs are increasing less rapidly than the number of eggs for samples with more than one layer of eggs. This indicates a curvilinear relationship between eggs and layers with apparent overestimation of layers of eggs. This phenomenon probably is the result of the patchy nature of egg deposition on the vegetation where several layers of eggs may be present but only on a portion of the individual plants. The diver then must estimate how many layers completely covering the plant this represents.

The parameter estimates for the vegetation types indicate that the three filamentous groups (sea grasses, other brown algae, and filamentous reds) are not markedly different from one another. Foliose reds are also probably not different from this group. The values for S_4 (kelp) and S_5 (rockweed) indicate that kelp has substantially fewer and rockweed substantially more eggs at a given percent cover and number of layers of eggs than do the other four groups. This is surprising because the three foliose types (rockweed, kelp, and foliose reds) have a larger surface area than the filamentous types so one would expect them to contain more eggs. However, there is a tendency for eggs on foliose vegetation to slough off and become lost during sampling which could explain the lower egg numbers in this group.

Predictions of egg numbers for all observations including those at less than one layer of eggs revealed some interesting anomalies in relation to actual egg counts. Any samples where the squared difference between the natural logarithm of the observed value and that predicted exceeded 3, equivalent to at least a fivefold difference in absolute value, were examined in detail. A total of 38 samples were found to be such outliers.

There are no universally acceptable means of objectively characterizing a residual as an outlier and removing it from a data set (Anscombe 1960). However, there is certainly the possibility for a better understanding of the structure of the data and behavior of the model by examining the larger deviations from the latter. Twenty-four of the samples were those with less than one layer of eggs. Of these, in 19 cases the predicted value greatly exceeded the observed value indicating lack of fit of the model at less than one layer of eggs. Some deviation from prediction would be expected here as the model was only fitted to observations with more than one layer of eggs but the consistent overestimation warranted further investigation of samples at less than one layer of eggs. An additional parameter was added to the model to attempt to remedy this problem, and its effects will be discussed below.

There were four outliers that included unusual species of vegetation present in restricted localities. Two samples were of a sea grass, *Phyllospadix* sp., and two of kelp, *Egregia* sp. In these cases the plants were much larger than the usual species of grasses and kelps and hence would be expected to contain unusually high egg numbers. We subsequently removed nine samples of these two species from the data set.

There were six other samples which probably consisted of unusually large or small plants. Although we have no measure of plant height the biomass of the vegetation plus eggs in the total sample is available. In comparison to other samples of the same percent cover and layers of eggs, four of these samples were much smaller and two were much larger in biomass than the average suggesting that an important variable is missing in these samples. This suggests that a useful variable for divers to measure if future sampling is considered would be the height of the vegetation in the quadrat as it is being sampled.

The dominant vegetation type estimated by the divers is that covering most of the quadrat rather than that containing most of the eggs. Quite often the samples contain two or more species of vegetation falling into two or more of the dominant vegetation groupings used here. Rarely the dominant vegetation type will contain fewer eggs than the minor vegetation

TABLE 2. Significance testing of the model to predict egg density including observations at less than one layer of eggs. (** Probability of greater $F < 0.01$; * probability of greater $F < 0.05$.)

	Model	Hypothesis tested (model tested)	Residual sum of squares	Degrees of freedom V_1	Degrees of freedom V_2	Partial F-statistic
a	$\log_e \alpha$	$\alpha=0$	2263.66	1	912	13884.70**
b	$\log_e \alpha+\beta\log_e L$	$\beta=0$ (b vs. a)	1031.05	1	911	1089.10**
c	$\log_e \alpha+\beta\log_e L+\delta\log_e L$	$\delta=\beta$ (c vs. b)	1008.70	1	910	20.16**
d	$\log_e \alpha+\beta\log_e L+\gamma\log_e P+S_i$	$\gamma=0$, (d vs. b) $S_i=0$	512.12	6	905	152.84**
e[a]	$\log_e \alpha+\beta\log_e L+\delta\log_e L+\gamma\log_e P+S_i$	$\gamma=1$ (e vs. f)	506.70	1	905	0.00
f[a]	$\log_e \alpha+\beta\log_e L+\delta\log_e L+\log_e P+S_i$		506.70			

[a]Where β occurs L refers to layers of eggs greater than one while where δ occurs L refers to layers of eggs less than one.

TABLE 3. Comparison of model predictions of thousands of eggs/m^2 with estimates from Haegele et al. (1979a) in parentheses for a range of vegetation density and spawning intensity (layers of eggs).

Filamentous	% cover of vegetation	Layers of eggs 0.5	1	3	5
Grasses	10	45 (52)	90 (64)	205 (112)	301 (159)
	50	224 (212)	449 (301)	1026 (647)	1506 (996)
	100	448 (391)	898 (588)	2052 (1380)	3010 (2194)
Other brown algae	10	46 (34)	92 (44)	210 (81)	308 (118)
	50	229 (423)	459 (487)	1048 (736)	1539 (982)
	100	458 (1248)	918 (1373)	2097 (1901)	3078 (2447)
Filamentous red algae	10	44 (48)	81 (75)	200 (178)	293 (279)
	50	218 (141)	437 (268)	999 (771)	1466 (1274)
	100	436 (223)	874 (463)	1997 (1450)	2933 (2448)
Foliose		**0.5**	**1**	**2**	**3**
Rockweed	10	34 (107)	69 (127)	115 (163)	157 (197)
	50	171 (244)	343 (394)	577 (690)	783 (977)
	100	342 (348)	685 (641)	1154 (1286)	1566 (1947)
Kelp	10	63 (36)	127 (54)	214 (90)	290 (125)
	50	317 (134)	635 (227)	1070 (413)	1451 (599)
	100	634 (235)	1270 (420)	2139 (797)	2901 (1196)
Foliose red algae	10	39 (13)	78 (27)	131 (54)	177 (80)
	50	194 (136)	388 (273)	653 (545)	886 (818)
	100	387 (371)	776 (740)	1306 (1481)	1772 (2231)

type. One outlier was such a sample where the substrate was mostly kelp but the eggs were all on the filamentous red algae. In general, this type of error would be expected to occur very rarely.

Lastly, there were three outliers for which no explanations were found. Overall 16 data points were subsequently deleted leaving 913 observations including those at less than one layer of eggs.

As noted above this model did not predict egg numbers very well at values of L less than one. We therefore examined the possibility of extending the model (equation 1A) by adding a parameter (δ) for observations at less than one layer of eggs. This is equivalent to equation 1A with:

$$Y = e^{\alpha} \cdot L^{\beta} \cdot P^{\gamma} \cdot e^{S_i} \cdot e^{\epsilon} \text{ for } L \geq 1$$

and

$$Y = e^{\alpha} \cdot L^{\delta} \cdot P^{\gamma} \cdot e^{S_i} \cdot e^{\epsilon} \text{ for } L < 1.$$

Adding δ after fitting the model with β alone resulted in a statistically significant reduction in residual variation indicating that δ was indeed significantly different from β and that this additional parameter for observations at less than one layer of eggs was justified for predictions at low spawn abundance (Table 2, model e). The other parameters α, β, γ, and S_i were also tested for significance with the additional observations and all were significantly different from their natural values except γ which was still not significantly different from one. The model now accounts for about 78% of the total variation in egg number.

The parameter estimates with 913 observations are similar

to those obtained using only 726 data points (Table 1). Adding the parameter δ resulted in a slight reduction in the value of β. The model also indicates that the number of eggs increases directly with number of layers if less than one and becomes curvilinear at layers of eggs greater than one as before. The parameter estimates for substrate types indicated the same differences between the filamentous and foliose groups. Rockweed was again higher, and kelp and foliose red algae lower, than the three filamentous groups which were all similar.

When this model was used for prediction there were still 17 outliers at less than one layer of eggs as opposed to 24 outliers with no parameter in the model for observations at less than one layer of eggs. No attempt has been made to interpret these remaining outliers further because large relative errors would be expected in these estimates. One should note that the egg numbers at such low egg layers are so small relative to those at several layers of eggs that the effect of even relatively large errors in estimation in these areas of sparse spawn will result in negligible effects on the overall estimate of mean egg density and overall egg number in a spawning.

However, there is an indication of a slight bias in the model predictions. The sum of the observed egg numbers for 913 observations was 967 628 while the sum of the predictions was 1 038 854, or a 7% positive bias. Whether this is significant could most usefully be tested by applying the model to observations taken in a spawning in relation to actual egg counts in that area. Unfortunately, this type of information is not presently available so the model cannot be adequately tested at this time.

In comparison to the estimates of Haegele et al. (1979a), the model predictions for filamentous vegetation are similar for all but other browns which is the vegetation type with the fewest observations (Table 3). In general, the model predicts more eggs in almost all instances for the filamentous vegetation types, in some instances markedly more. For the foliose groups, rockweeds and foliose reds, predictions are remarkably similar to those of Haegele et al. (1979a) while for kelps there is no consistent pattern to the discrepancies.

On the whole, although the two sets of predictions are not very disparate, we have been able to condense the earlier results into a comprehensive model of only eight parameters in contrast to the multitude required previously which should facilitate and expedite the estimation of egg numbers for stock assessment.

A number of other questions and dilemmas remain. How significant are the effects of inter-diver, area, year, and quadrat size variation on the parameters of the model and hence on estimates of egg density? Obviously, given the immediacy of management requirements, the available results should be applied to stock assessments on an interim basis but these remaining concerns should be addressed at the earliest opportunity.

Acknowledgments

We particularly thank C. W. Haegele for numerous discussions and assistance in evaluating outliers. Dr D. Hay provided the impetus for this work and Dr A. S. Hourston constructively critiqued an early draft of this manuscript.

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Temperature and Scale Size Errors in the Use of [^{14}C]Glycine Uptake by Scales as a Growth Index[1]

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SMAGULA, C. M., AND I. R. ADELMAN. 1982. Temperature and scale size errors in the use of [^{14}C]glycine uptake by scales as a growth index. Can. J. Fish. Aquat. Sci. 39: 1366 – 1372.

Measurement of glycine uptake by largemouth bass (*Micropterus salmoides*) scales at several acclimation (14, 20, and 26°C) and incubation (14–32°C, 3°C increments) temperatures revealed significant interaction between these factors ($P < 0.005$). Therefore, comparison of uptake data from several acclimation groups could not be made at any arbitrary "standard" incubation temperature without introducing error. An error-free scale incubation criterion was tentatively proposed as optimum incubation temperature for each fish acclimation temperature, determined from a laboratory response surface model. Incubation temperature of maximum uptake was associated with temperature of maximum body growth of bass which suggests that uptake may be integrated with growth. Use of scale weight to express uptake relative to scale size will probably introduce systematic error, and scale area should provide the "best" measure for normalization of uptake data. Glycine uptake by 14, 20, and 26°C acclimated scales was linearly related to body growth on a $\log_e - \log_e$ basis ($P < 0.05$); however, within acclimation groups significant correlation was not noted.

Key words: acclimation temperature, incubation temperature, response surfaces, largemouth bass, *Micropterus salmoides*

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Des mesures d'absorption de glycine par les écailles d'achigans à grande bouche (*Micropterus salmoides*) à plusieurs températures d'acclimatation (14, 20 et 26°C) et d'incubation (14–32°C, par augmentations de 3°C) indiquent une interaction significative entre ces facteurs ($P < 0{,}005$). C'est pourquoi on ne peut comparer les données sur l'absorption parmi plusieurs groupes d'acclimatation à une température d'incubation «standard» arbitraire, sans introduire une erreur. Nous proposons provisoirement un critère d'incubation des écailles dépourvu d'erreur comme température d'incubation optimale pour chaque température d'acclimatation des poissons, établi d'après un modèle de laboratoire de surface de réponse. La température d'incubation d'absorption maximale est associée à la température de croissance du corps maximale de l'achigan, ce qui donne à penser que l'absorption peut être intégrée à la croissance. L'utilisation du poids des écailles pour exprimer l'absorption en relation avec la grandeur de l'écaille introduira probablement une erreur systématique. La superficie de l'écaille devrait fournir la «meilleure» mesure de normalisation des données d'absorption. L'absorption de glycine par des écailles acclimatées à 14, 20 et 26°C est en relation linéaire avec la croissance corporelle sur base $\log_e - \log_e$ ($P < 0{,}05$); cependant, nous n'avons pas noté une corrélation significative à l'intérieur des groupes d'acclimatation.

Received August 26, 1981
Accepted June 14, 1982

Reçu le 26 aout 1981
Accepté le 14 juin 1982

A physiological index of fish growth has been introduced by Ottaway and Simkiss (1977a, b, 1979) as in vitro uptake of [^{14}C]glycine by scales. Despite its promise, glycine-uptake methodology cannot be applied prior to determination of a proper measure of scale-reactive potential (size) and also prior to determination of a proper scale incubation temperature criterion. These criteria are necessary for normalization of uptake data and for statistical comparisons among groups of fish acclimated to different temperatures.

Ottaway and Simkiss (1977a) have associated glycine uptake by scales with body growth, using scale weight to express uptake on a relative basis. They also used a temperature

[1]Paper No. 11876, Scientific Journal Series, Minnesota Agricultural Experiment Station, Saint Paul, MN 55108, USA.

Printed in Canada (J6606)
Imprimé au Canada (J6606)

coefficient (Q_{10}) apparently derived from a single acclimation group, to adjust uptake data measured at different acclimation and incubation temperatures to a "standard" 14°C incubation level. Since Ottaway (1978, fig. 4) reported that uptake varied over different scale regions, being concentrated on the anterior and posterior dermal-covered margins, but also present on epidermis-covered central scale surfaces, it appears that reactive potential of scales is related to scale area and edge, not to scale weight. Therefore, use of scale weight to normalize uptake data will introduce bias against larger scales as weight is not a linear function of area or circumference. Bias will occur as a result of actual reactive potential (amount of area and edge) increasing at a lesser rate than the employed measure of reactive potential (weight) as scale size increases. Hence, uptake normalized to scale weight will underestimate uptake by "larger" scales relative to uptake by "smaller" scales.

Adelman (1980) reported an interaction between acclimation temperature of fish and incubation temperature of scales. Thus, when several acclimation groups of fish are compared, no arbitrarily designated "standard" incubation temperature for uptake measurement is valid. Objectives of this study were as follows: (1) to determine an expression of uptake relative to scale size which was not biased with respect to the manner in which glycine uptake occurs on scales; (2) to determine an incubation temperature model for uptake by scales which was not biased with respect to acclimation state; and (3) to examine the uptake : growth relationship among age 0 largemouth bass (*Micropterus salmoides*).

Materials and Methods

To establish the relationships between radius, circumference, area, and weight of scales, scales ranging in weight from 1 to 60 mg were taken from 125 bass ranging in age from 0 to VI and weight from 0.01 to 1.6 kg. Measurements were made with a digitizing pad interfaced with a computer.

For the experiment to determine temperature effects, age 0 largemouth bass were collected by electrofishing in Lake Rebecca, Hennepin County, Minnesota. Bass were transported to the laboratory and held in 330-L flow-through tanks at 23°C and constant photoperiod (LD 12 : 12) for 1 wk. Ten bass were then randomly assigned to each of three 330-L tanks. Temperature was adjusted from 23°C to experimental acclimation levels of 14, 20, and 26°C (0.5°C · d^{-1}). Bass were held for 4 wk at these temperatures, then anesthetized (tricaine methanesulfonate), weighed (g), measured (total length in mm), and uniquely marked by liquid nitrogen branding. Mean initial lengths were 122, 125, and 127 mm for 14, 20, and 26°C temperatures, respectively. During experiments 50 fathead minnows (*Pimephales promelas*) (mean weight = 1.2 g) were introduced daily to tanks containing bass. Uneaten minnows were removed daily and their numbers and weights were recorded. Daily consumption never exceeded 38 minnows; therefore, it is assumed that bass were being fed ad libitum. Bass were remeasured after 9 d and also at the termination of the experiment (after 34, 35, and 36 d for the 14, 20 and 26°C acclimated bass).

Uptake determinations were begun at 11 : 00 on each of three consecutive days for the three acclimation groups. Scales were removed from the left body side in the second scale row below the lateral line, nearest the tip of the posteriorly extended pectoral fin. Regenerated scales were discarded. At seven incubation temperatures (14–32°C, 3°C increments) four scales from each fish were incubated for 2 h, each in a separate [^{14}C]glycine concentration (5, 15, 30, and 62.5 μmol/L) dissolved in 0.4 mL of 0.7% physiological saline (Shuttleworth 1972). Control scales were immersed in 60°C water to determine uptake not associated with active processes. Glycine uptake by controls ranged from 0.5 to 1.3 pmol · mm^{-2} · 2 h^{-1} and were subtracted from uptake by experimental scales. Uptake by scales was converted to pmol by the method of Adelman (1980). Order of incubation treatments and order of fish sampled at each incubation level were randomly determined. After incubation in glycine, scales were placed in a saline rinse for 2 h, then dried to a constant weight at 60°C and weighed to the nearest microgram. Scales were then digested in 0.4 mL of Soluene®, and radioactivity of the labeled glycine was counted in a manner identical with that of Adelman (1980).

An uptake–growth relationship was analyzed by least squares linear regression. The variance of uptake data was proportional to mean uptake values. Therefore, uptake data were transformed to natural logarithms to provide uptake variances independent of mean uptake values and to meet homogeneity of variance assumptions for regression and for analysis of variance of the effects of acclimation and incubation temperature and glycine concentration on uptake.

Combined acclimation × incubation effects on uptake were modeled using least squares polynomial response surfaces fit to the laboratory data. Adequacy of models was analyzed by criteria of parsimony, values of r^2 (adjusted for the number of predictors), and Mallow's $C(p)$ statistic (Daniel and Wood 1971). The $C(p)$ statistic measures the bias in prediction of an empirical model with $C(p)$ less than or equal to the number of predictors in the model indicating that the model is not biased in prediction and is a candidate for selection (Weisberg 1980).

Results

Scale Size Effects

Ottaway (1978) reported that a variety of cell types are sampled when a scale is removed from a fish. During sampling, scale-forming cells of dermal origin are exposed on the anterior margin while both epidermal and dermal cells remain on the posterior margin. Scale weight would a priori be a poor choice as the parameter of scale size for normalizing uptake data because scale-forming cells are superficially distributed (Ottaway 1978), and hence, most of the weight of a scale would not contribute to the reactive surface. Empirically, Ottaway's (1978, fig. 4, table 2) results also preclude use of scale weight, because area and edge were the parameters of scale size which reflected regional differences in the uptake potential of scales. Obviously, weight of scales will correlate with amount of area and edge present; however, the relationship will not likely be linear. For bass scales (ages 0–VI) the weight–area relationship was nonlinear (Table 1).

The percent error incurred, when expressing uptake relative

TABLE 1. Nonlinear relationships between radius (R), circumference (C), area (A), and weight (W) of circles, largemouth bass scales, and physical bodies in general. Radius of bass scales (ages 0–VI) was measured on the second dorsal scale radius. Bass scales ranged from 1 to 60 mg (N = 125). In the general area to weight relationship, K is a constant specific to the physical body in question. Nonlinear relationships were linearized by the natural logarithm transformation. Confidence limits (95%) are indicated as (±).

Object		Correlation
Circle	Bass scales	r^2
$C = 2\pi R$	$C = (1.60 \pm 0.002)\pi R$	0.98
$\log_e C = 1.27 + 0.50 \log_e A$	$\log_e C = (1.17 \pm 0.006) + (0.51 \pm 0.0006) \log_e A$	0.99
$\log_e R = -0.57 + 0.50 \log_e A$	$\log_e R = (-0.53 \pm 0.009) + (0.52 \pm 0.001) \log_e A$	0.99
Bodies in general[a]		
$\log_e W = K + 1.5 \log_e A$	$\log_e W = (-2.11 \pm 0.08) + (1.42 \pm 0.004) \log_e A$	0.98

[a]From the relationship that $W \cong L^3$, where L is a linear measurement and the relationship that $A \cong L^2$.

to scale weight (W) despite reactive potential of scales being a function of surface area (A), can be quantified using the empirical relationship between weight and area of bass scales contained in Table 1. Taking the antilogarithm of the scale weight:area relationship and solving for A yields:

$$A = 5.4W^{0.7}. \quad (1)$$

The difference between scale weight and area in an expression of uptake (uptake per unit of scale size) would be:

$$W - A. \quad (2)$$

Expressing this difference relative to scale area yields:

$$\frac{W - A}{A}. \quad (3)$$

Subtracting 1.0 from (3) and multiplying the new expression by 100 yields the percent error incurred when expressing uptake relative to scale weight rather than scale area (4):

$$100 \cdot \frac{W - A}{A} - 1.0. \quad (4)$$

Note, when A is expressed in units of W (1), expression (3) will always be greater than 1.0. Therefore, subtracting 1.0 and multiplying by 100 yields the percent by which scale weight exceeds scale area in an uptake expression. That is, a positive value of (4) indicates that uptake is being normalized to a larger than correct scale size. The percent of this error is also given by (4). Substituting for A in expression (4) yields a formula for the percent error across a range of scale sizes, for the case when uptake is expressed relative to scale weight rather than scale area.

$$100 \cdot \left(\frac{W - 5.4W^{0.7}}{5.4W^{0.7}} - 1\right). \quad (5)$$

Simplifying (5) yields:

$$100 \cdot \left(\frac{W^{0.3}}{5.4} - 2.0\right). \quad (6)$$

Note that error is an increasing function of scale size, that is, the difference between weight and area expressions of scale size increases as scale size increases. Therefore, uptake by "larger" bass scales would be underestimated relative to uptake by "smaller" scales if uptake were normalized to scale weight.

Similar to weight and area, other size parameters of bass scales maintain nonlinear relationships (Table 1). The relationships in Table 1 are empirical relationships obtained from measurements of a range of bass scales and the same relationships for perfectly circular scales. For bass this indicates that to avoid bias when expressing uptake relative to scale size, regional differences in uptake on scales should be considered. Equations in Table 1 indicate that the amount of edge relative to area on bass scales decreases as scale size increases. Therefore, larger scales will have correspondingly less reactive edge relative to their area. To account for this differential, absolute uptake (U_{abs}) could be normalized to both edge (C) and area (A) as:

$$\frac{U_{abs}}{\beta_1 A + \beta_2 C} = \frac{U_{abs}}{\beta_1(5.4W^{0.7}) + \beta_2(7.6W^{0.36})}. \quad (7)$$

β_1 and β_2 are coefficients determined from the area-edge ratio (α):

$$\alpha = \frac{A}{C} = \frac{5.4W^{0.7}}{7.6W^{0.36}} = 0.71W^{0.34}. \quad (8)$$

Under the constraint that area and edge sum to 100%, β_1 and β_2 are:

$$\beta_1 = 1 - \beta_2 \quad (9)$$

$$\beta_2 = \frac{1}{\alpha + 1}.$$

Unfortunately, equations (6) and (8) are sensitive to units of measurement as different results would be obtained if scale weight were expressed as milligrams versus micrograms (by a factor of 10^3). Therefore, in this approach uptake data should be expressed in biologically relevant units with respect to the dimensions of scale-forming cells, being on the order of a few microns. Scale weight expressed in units relevant to the weight of scale-forming cells would be at least on the order of 10^3–10^4 for age 0–VI bass. This order of magnitude causes α to become large and hence β_2 to approach zero. Simply, in cellular dimensions a bass scale consists mostly of

area rather than edge. However, equation (8) is somewhat unrealistic in that it relates area, which is a two-dimensional parameter, to circumference, which is a one-dimensional parameter. Hence, as scale size increases, area overwhelms edge as a result of area increasing approximately as the square of edge. To be biologically most meaningful, the inner surface of a scale should be differentiated from a two-dimensional border containing osteoblasts, not a one-dimensional edge was considered in equation (8). If the width of the growing margin were a constant percentage of the scale radius, then no differentiation between the central area and the growing margin would be needed, as the area-edge ratio would be constant. However, if the width of the growing edge were a function of scale size, then the previous approach would apply (Eq. 7), with edge now being defined as a two-dimensional border of the scale surface containing osteoblasts. It is likely that the width of growing scale edge is not a constant percentage of the scale radius, as both body and scale growth are relatively greater in younger fish (smaller scales). This would suggest that the width of the growing edge will be a greater percentage of the scale radius in smaller fish, which would compound the previously discussed differentials.

At the present time uptake should be normalized to scale area prior to further determination of regional differences in uptake potential associated with different cell types. In the present study all data are normalized to scale area; that is, measurements of scale weight were transformed to scale area using the empirical scale weight−area relationship developed from age 0 to VI bass scales. Use of the weight−area equation allowed uptake to be normalized to area without making the more tedious direct measurement of scale area.

A less complex procedure for normalization of uptake data to scale size would be merely to express uptake relative to the $\log_e$ of scale weight. From Table 1 and in general it is obvious that taking the logarithm of an exponential weight−area relationship will linearize it. However, if this were done, uptake would not be expressed in direct units of scale size. The previous more-involved method of normalization was adopted in this study to allow comparison with previous studies and to retain uptake expressions in untransformed units of scale size prior to resolution of biological questions concerning regional differences in uptake activity of scales.

Uptake−Growth Relationship

Growth rate of bass increased with increasing acclimation temperature (Fig. 1A). The relationship between uptake and body growth was not linear across acclimation groups. Relative to 14 and 20°C acclimated bass, increases in uptake by the rapidly growing 26°C acclimated bass were not proportional to body growth (Fig. 1A). However, on a $\log_e$−$\log_e$ basis uptake was linearly related to growth ($r = 0.86$, $P < 0.01$) (Fig. 1B). Interestingly, daily food consumption by 26°C acclimated bass decreased by 40% for 3 d prior to uptake measurement while consumption by bass at 14 and 20°C remained at stable levels. Growth rate of 26°C acclimated bass may have been depressed during uptake measurement relative to 14 and 20°C acclimated bass to account for the less-than-proportional increases in uptake by bass at 26°C (Fig. 1).

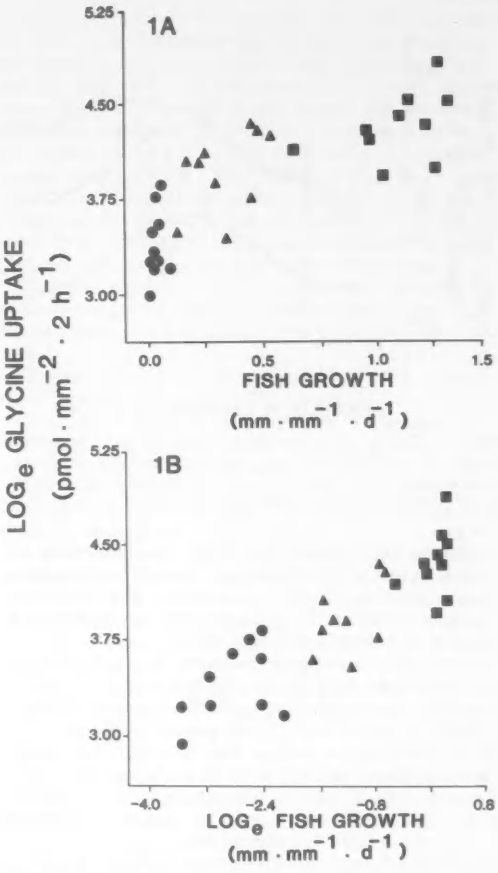

FIG. 1. Relationship between glycine uptake by scales and relative growth in length (A) or the natural logarithm of relative growth in length (B). Uptake is expressed at optimum incubation temperature. Note that growth rate and uptake increase with increasing acclimation temperature. (● = 14°C, ▲ = 20°C, ■ = 26°C acclimated largemouth bass).

Temperature and Glycine Concentration Effects

Analysis of variance indicated that both acclimation and incubation temperature significantly affected uptake (U) ($P < 0.005$, Fig. 2). Glycine concentration (G) effects were also significant ($P < 0.005$), with uptake by bass scales being linearly related to glycine concentration (5−62.5 μmol/L), with a regression equation of $\log_e U = 2.58 + 1.74G$ ($r = 0.96$, $P < 0.01$). Concentration did not interact with either acclimation or incubation temperature ($P > 0.05$), which allowed uptake to be pooled across concentrations for each fish for more precise estimation of acclimation and incubation effects (Fig. 2).

Significant interaction occurred between acclimation and

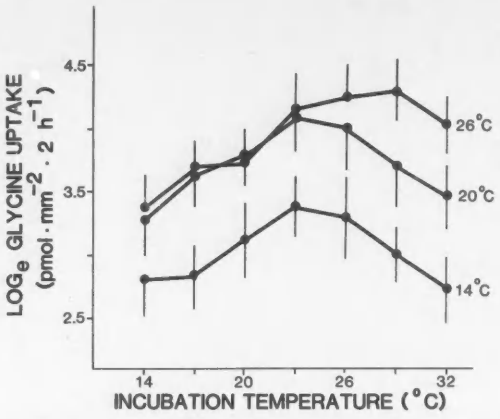

FIG. 2. Glycine uptake (geometric means) by age 0 largemouth bass scales acclimated to 14, 20, and 26°C across a 14–32°C incubation range. Uptake (± 95% C.L.) was averaged across several glycine concentrations for scales from each fish ($N = 10$).

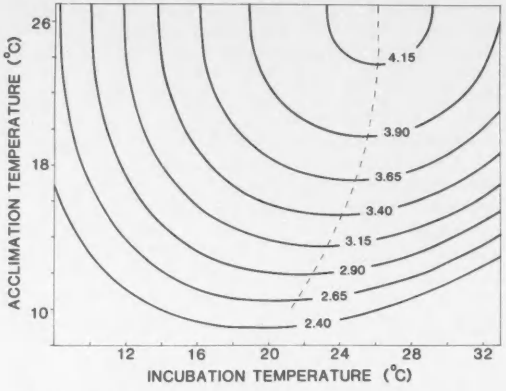

FIG. 3. Isopleths of the response surface fit to glycine-uptake data ($\log_e$ pmol · mm^{-2}) from age 0 largemouth bass scales in the acclimation × incubation temperature plane. Isopleths occur at 0.25 intervals. The broken line defines the incubation temperature of maximum uptake for each acclimation level.

incubation temperatures ($P < 0.005$, Fig. 2). Uptake increased at each acclimation temperature with warmer incubation, reaching a maximum in the 23–29°C incubation region; however, beyond 29°C incubation, uptake decreased for each acclimation group ($P < 0.05$).

At all incubation temperatures, uptake by rapidly growing 26°C acclimated bass and moderately growing 20°C acclimated bass was higher than uptake by slow-growing 14°C acclimated bass ($P < 0.05$). However, significant differences between uptake by scales from 26 and 20°C acclimated fish were present only at 29 and 32°C incubation ($P < 0.05$). Averaged over all other factors, uptake at 23–29°C incubation was higher than uptake at 14–20°C incubation ($P < 0.05$). At 23°C incubation, scales from 14 and 20°C acclimated fish had higher uptake when contrasted against all other incubation temperatures ($P < 0.05$), which was not true for scales from 26°C acclimated fish (Fig. 2). The interaction between acclimation and incubation temperatures can be noted in the shift in incubation temperature of maximum uptake to warmer levels for scales from warmer acclimated fish.

TEMPERATURE STANDARDIZATION

Polynomial response surface models were fitted to the laboratory data to establish a procedure for normalizing uptake (U) by different acclimation groups of fish to a standard incubation temperature for statistical comparisons. This was necessary owing to the acclimation × incubation interaction, as uptake at any arbitrary "standard" incubation temperature will be a varying percentage of maximum uptake at the optimum incubation temperature, depending on previous thermal history.

Models fitted included terms and cross products in acclimation (A) (to quadratics) and incubation (I) (to cubics). Higher-order terms were not fitted to the final model because Alderdice (1972) suggested that such terms may not be relevant for most biological processes, and in the present study were found to account not significantly for unexplained variance in the uptake-temperature relationship. Subsets of the full model were obtained using the "screen" algorithm (Weisberg 1977), which efficiently selects models that satisfy previously stated criteria. The following model was clearly "best": $\log_e U$ (pmol · mm^{-2}) $= 1.065 + 0.0136AI - 0.000259A^2I - 0.000086I^3$ where r^2 (adjusted) $= 0.94$, $N = 21$, and $P < 0.01$ for all terms. The model describes in vitro uptake (U) of glycine by bass scales as an interaction between acclimation and incubation temperature (AI) which produces higher uptake at warmer acclimation and incubation temperatures. However, the negative A^2I term in the model embodies the asymmetry of the uptake response, in that increases in uptake decreased as temperatures of incubation and especially acclimation (A^2) increased. The negative I^3 term indicates that uptake by all acclimation groups decreased rapidly at the warmest incubation temperatures. Isopleths of the uptake surface illustrate the asymmetrical nature of uptake by bass scales (Fig. 3).

The incubation ridge of the surface (incubation temperature of maximum uptake for a given acclimation temperature) and the factor levels that produced maximum uptake on the surface were obtained by forming the gradient vector (∇) of the empirical model describing the uptake surface (U).

$$(10)\quad \nabla U(A,I) = \frac{\partial U}{\partial A}(A)\,\vec{i} + \frac{\partial U}{\partial I}(I)\,\vec{j}$$

where

$$\frac{\partial U}{\partial A} = (0.00136)I - (0.0000518)AI$$

$$\frac{\partial U}{\partial I} = (0.00136)A - (0.0000259)A^2 - (0.0000258)I^2.$$

Setting $\frac{\partial U}{\partial A}$ and $\frac{\partial U}{\partial I}$ to zero and solving the equations simultaneously yields the acclimation and incubation temperatures of maximum uptake on the surface (A = 26.3°C, I = 26.3°C). Setting $\frac{\partial U}{\partial I}$ to zero and solving for I yields an equation for the incubation ridge (I^*).

(11) $I^* = (52.819A - 1.006A^2)^{0.5}$.

Equation (11) indicates that increments of optimum incubation temperatures increase approximately as the square root of acclimation temperature. In actuality, our data are for discrete values of A and I and equation (11) assumes continuous behavior between our experimental values of A and I. Curvature of the incubation ridge indicates that warmer acclimation produced increasingly smaller increases in optimum incubation temperature, which embodies thermal constraints on physiological adaptation of fish. Similarly, decreases in uptake at high incubation temperatures are more rapid for cooler acclimated bass (Fig. 3).

Equation (11) is valuable in practical applications for adjusting glycine uptake from experimental incubation temperatures to uptake at optimum incubation levels. The standardization factor Ω for a given acclimation group would be:

(12) $\Omega = \frac{U_{I^*}}{U_I}$ where U_{I^*} = predicted uptake from the response surface at the optimum incubation temperature and U_I = predicted uptake from the response surface at the experimental incubation temperature.

Therefore, uptake "standardized" to an optimum incubation temperature is:

(13) $U_{adj} = \Omega U_{exp}$ where U_{adj} = uptake adjusted to an optimum incubation temperature and U_{exp} = uptake at the experimental incubation temperature.

The response surface model allows standardization of uptake measured at different acclimation and incubation temperatures to an optimum incubation temperature for each acclimation group. Optimum incubation temperature is probably the most appropriate incubation criterion, as this incubation temperature would not arbitrarily limit uptake among differentially acclimated fish (scales). Similar equations could be used to adjust empirical uptake data to any desired incubation temperature.

Discussion

Acclimation effects on metabolism and growth of bass found in the present study agree with the results of Beamish (1970) and Niimi and Beamish (1974), respectively, in which maximum scope for activity occurred at 30°C and maximum growth occurred at 25°C. Other studies (Strawn 1961; Lee 1969; Coutant and Cox 1976) have also found the 25–30°C temperature range to be optimal for bass growth. In our study the 25–30°C range also contained the acclimation (26.3°C) and incubation (26.3°C) temperatures that produced maximum uptake by bass scales on the uptake response surface. The 25–30°C region has also been reported to be optimal for digestion (Beamish 1972) and nitrogen assimilation (Savitz et al. 1977) in bass. It appears that optimal thermal conditions for uptake by bass scales correlate with temperatures that produce maximum growth efficiency in this species. Similarly, results from Adelman (1980) suggest that incubation temperature optima for uptake by scales correlate with thermal optima for body growth in white sucker (*Catostomus commersoni*) (~24°C), yellow perch (*Perca flavescens*) (~23°C), and bluegill (*Lepomis macrochirus*) (~30°C). Correlation between thermal optima for glycine uptake by scales and body growth suggests that processes associated with uptake may be associated with general growth processes.

Variability was noted in the present study in the relationship between glycine uptake by scales and body growth. Factors which may affect an uptake–growth relationship may be related to:

1) Differentials in endogenous energy supplies of scale cells;

2) Hormonal influences on amino acid incorporation into cells;

3) Temporal phases in scale growth.

Firstly, nutritional state of scale cells may be important for uptake of glycine as Boge et al. (1979) reported that glycine uptake by intestinal cells of rainbow trout (*Salmo gairdneri*) was an active process and a function of endogenous energy (glycogen) present in cells. Because energy must be expended by cells to obtain glycine from solution, and assuming that scale cells at higher nutritional levels (from faster growing fish) will be able to take up more glycine per unit time, then higher uptake by scales from faster-growing fish may occur through whole fish nutritional state being correlated with cellular nutritional levels. This suggests that in vitro uptake by scales may be considered as a test to determine scales having highest energy reserves.

Secondly, hormonal state may also affect uptake by scales because hormones regulate processes associated with body growth (Donaldson et al. 1979). On a cellular level, transfer of nutrients, metabolites, and ions across membranes is necessary for growth. Talwar et al. (1975) postulated that the growth-promoting effect of the pituitary growth hormone (GH) is related to facilitation of uptake of amino acids across cell membranes, which suggests that GH could affect in vitro uptake by scales. Similarly, Tashima and Cahill (1968), Thorpe and Ince (1974), and Inui et al. (1978) have associated insulin levels with increased uptake of amino acids (including glycine) into muscle tissue of oyster toadfish (*Opsanus tau*), northern pike (*Esox lucius*), and Pacific hagfish (*Eptatretus stouti*), respectively. Similar effects of hormonal state on uptake by scales and fish growth suggest that experiments should be carried out to investigate growth phenomena occurring simultaneously on cellular and organismal levels.

Thirdly, Fouda (1979) has suggested that growth and calcification of fish scales is a four-phase process, consisting of:

1) Production of an osteoid matrix by marginal osteoblast cells;

2) Calcification of the above matrix by inner osteoblasts;

3) Growth of the underlying fibrillary plate by deposition of successive lamellae of collagen below the calcified osteoid zone and similar deposition below the entire fibrillary plate;

4) Arrangement of calcified particles into successive bands.

Duration and overlap of growth phases may affect measurement of glycine uptake by scales. Growth phases 1 and 3 would a priori induce higher uptake because demand for glycine would be higher during deposition of collagen on the osteoid matrix and fibrillary plate. Phases in scale growth may be the proximate cause of diel fluctuations in uptake reported by Ottaway (1978).

In practical applications of glycine-uptake methodology, such as in seasonal growth studies on natural populations, expression of glycine uptake at optimum incubation temperature should theoretically provide the best standard. This criterion would not limit uptake by scales from differentially acclimated fish. However, at this time it is uncertain if uptake at optimum temperature or at the fish acclimation temperature would result in a higher correlation with the actual growth rate of the fish. Adelman (1980) suggested the practical alternative of scale incubation at the fish acclimation temperature. The response surface model, parameterized from laboratory data, provides a means of adjusting to any desired incubation temperature from any actual incubation temperature.

Acknowledgments

This work was supported by the University of Minnesota Agricultural Experiment Station and the University of Minnesota Computer Center. We are extremely grateful to P. A. Cochran for assistance in conducting laboratory experiments, to G. R. Spangler and D. F. Alderdice for suggested improvements in the manuscript, and to Kristin Lee Smagula for graphics and general moral support.

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Problems of Species Identity in the Lake Ontario Sculpins *Cottus bairdi* and *C. cognatus*[1]

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The sculpins *Cottus bairdi* and *C. cognatus* are difficult to distinguish in Lake Ontario because intermediate specimens are present and because *C. bairdi* in the lake is more variable than *C. bairdi* from the surrounding drainage basin. Our evidence indicates that intermediates are probably the products of hybridization between *C. bairdi* and *C. cognatus*. The greater variability of *C. bairdi* in Lake Ontario may be due to subspecies differences, but we suggest that backcrossing of *C. bairdi* with hybrids would also account for this phenomenon.

Key words: sculpin, Lake Ontario, taxonomy, hybridization

GODKIN, C. M., W. J. CHRISTIE, AND D. E. MCALLISTER. 1982. Problems of species identity in the Lake Ontario sculpins *Cottus bairdi* and *C. cognatus*. Can. J. Fish. Aquat. Sci. 39: 1373 – 1382.

Les chabots *Cottus bairdi* et *C. cognatus* sont difficiles à séparer dans le lac Ontario par suite de la présence d'intermédiaires et aussi parce que *C. bairdi* dans le lac est plus variable que les sujets habitant le bassin hydrographique avoisinant. D'après nos données, les intermédiaires sont probablement le produit d'hybridation entre *C. bairdi* et *C. cognatus*. Il se peut que la plus grande variabilité de *C. bairdi* dans le lac Ontario soit due à des différences sous-spécifiques, mais nous avançons l'hypothèse qu'un rétrocroisement de *C. bairdi* avec des hybrides pourrait également expliquer ce phénomène.

Received October 7, 1981
Accepted June 15, 1982

Reçu le 7 octobre 1981
Accepté le 15 juin 1982

SCULPINS are a major food item for predators like lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*) and, as such, are an important component of the Great Lakes fish communities. In the past, research emphasis was mainly applied to species of economic importance, and because of this, sculpin biology in the Great Lakes is not well understood. The investigation on which this paper was based was undertaken as an effort to fill some of the more obvious life history information gaps. It was first necessary to clarify some taxonomic questions and this report contains the results of that inquiry. A subsequent report will deal with life histories and productivity.

We found it difficult to distinguish between the species *Cottus bairdi* and *C. cognatus* using the characters recommended by McAllister (1964). Most specimens fell readily into one species or the other, but some displayed varying degrees of intermediacy or mixture of diagnostic characters. Here we refine identification of the two species and try to resolve the nature of problematical specimens, whether extreme variants or the result of hybridization.

This question is important because hybridization has not been documented between species of *Cottus* in North America (Schwartz 1981). In a letter[2] to Thomas Savage in 1958, the late C. L. Hubbs expressed his suspicions as follows: "Many times while working intensively with the fishes of Michigan, handling material of *Cottus* from streams where *bairdii* and *cognatus* occur together, I wondered whether hybrids were included. Several times I leaned to that interpretation, but could never find convincing evidence. Nor have I found other evidence of natural hybridization within the family, either in other freshwater localities, or in marine localities, where related species often live together."

The suspicion emerged more recently when K. M. Howse (in Lee et al. 1980) stated that variation in *C. princeps* suggested hybridization with *C. klamathenis*, *C. tenuis*,

[1]Contribution No. 81-10, Ontario Ministry of Natural Resources, Fisheries Branch, Maple, Ont. L0J 1E0

[2]We are indebted to Laura C. Hubbs and the Hubbs Library, Scripps Institution of Oceanography, for locating and providing access to this communication.

Printed in Canada (J6659)
Imprimé au Canada (J6659)

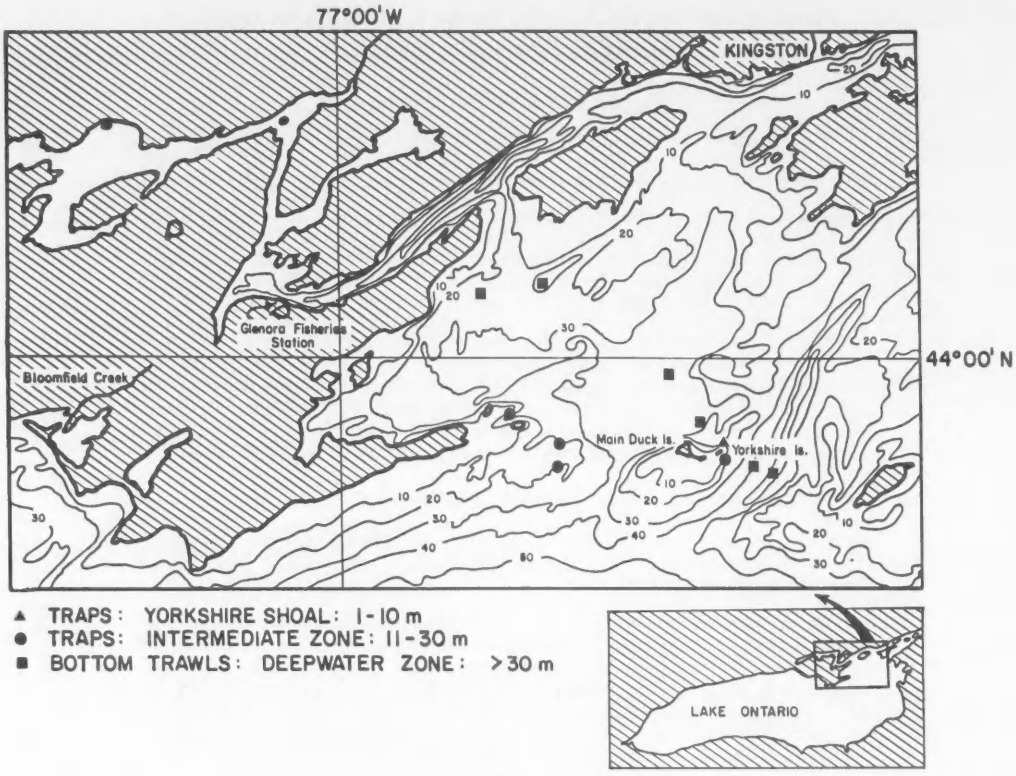

FIG. 1. Collection sites.

or an undescribed species. In Scandinavia, Nyman and Westin (1968) and Andreasson (1969) were more positive in their reports of hybridization between *Cottus gobio* and *C. poecilopus* in areas subject to disturbance by hydroelectric impoundments.

Materials and Methods

Collections were made in the eastern basin of Lake Ontario (Fig. 1) using bottom trawls in areas of soft bottom and traps where rocky substrate precluded the use of trawls. A few additional specimens were collected at trap sites by divers, and some were collected from Yorkshire Shoal at a 1-m depth by seining. Sculpins from Lake Ontario ranged in size from 17 to 122 mm total length, but only specimens over 35 mm were used in this study. All lengths are total lengths, unless otherwise noted. Sculpins from Bloomfield Creek, collected by seining and electrofishing, ranged from 23 to 107 mm in length.

Measurement methods for meristics and morphometrics followed McAllister (1964). He compared the effectiveness of the 12 characters listed in Table 1 for *C. bairdi* and *C. cognatus* for separating the two species. His samples were taken from collections made from northern Quebec through Ontario and the Great Lakes. He found that the ratio of caudal peduncle length:postorbital distance (measured from eye to bony edge of operculum) was the only character which gave 100% separation levels. We found that this character had a number of measurement limitations, and this should be borne in mind when considering the results of the statistical analysis. First, the measurement error within Lake Ontario specimens was high; estimated at ± 10.8% (± 11.6% for small specimens). Second, the postorbital length in the occasional specimen differed on left and right sides. Thirdly, it was found that, in measuring the caudal peduncle length, torn anal fins resulted in a greater measurement error in the case of *C. bairdi* than in *C. cognatus*, because of differences in the way the soft tissue of the anal fin attaches to the body (Fig. 2). Specimens with torn anal fins were therefore not included in the analysis.

Estimates of Separation Levels Between Species

We used several methods to measure species identity and assess numbers of intermediates in each population. First we compared Lake Ontario specimens with the populations reported on by McAllister (1964). Preliminary studies suggested that cottid species distribution in Lake Ontario is re-

TABLE 1. Comparison of separation levels for diagnostic characters in *Cottus bairdi* and *C. cognatus*, given as the percentage of specimens separable by each character.

Character	Comparison, %		
	McAllister (1964)	Bloomfield Creek vs. 42-m depth in Lake Ontario	1-m depth in Lake Ontario vs. 42-m depth in Lake Ontario
Caudal peduncle length/post orbital distance[a]	100	81	66
Total number of pelvic[b] + pectoral[b] + anal[c] + dorsal[c] rays	96	91	84
Last rays of dorsal double or single	95	83	83
Number of pelvic rays[b]	94	99	99
Total number of pelvic[b] + pectoral[b] + anal[c] rays	94	87	78
Palatine teeth[b]	94	69	52
Number of anal rays[c]	88	81	76
Last rays of anal double or single	86	90	88
Number of dorsal rays[c]	79	78	73
Length of longest and ray/standard length	79	78	54
Number of pectoral rays[b]	79	68	70
Number of postmaxillary pores[b]	74	67	66

[a]Measured from eye to bony edge of operculum.
[b]Determined on left side.
[c]Each of last two dorsal and anal rays counted, even if close basally.

lated to depth; *C. bairdi* being a shallow-water species, whereas *C. cognatus* extends as deep as our deepest collections at 73 m. Using McAllister's 12 characters we compared samples of 32 *C. bairdi* from a 1-m depth in Lake Ontario with 50 *C. cognatus* from a 42-m depth, and separation levels were assessed as the percentage of specimens separable by each character. Additional comparisons were made between 50 *C. bairdi* from Bloomfield Creek compared with the *C. cognatus* sample from Lake Ontario at the 42-m depth.

Estimates of Variation Within Different Populations

The above comparison was limited by the small sample of specimens from 1 m in Lake Ontario, so we attempted to quantify the differences between stocks using four characters considered simultaneously. This procedure enabled us to examine large numbers of specimens over a wide range of collection sites, and so, in addition to providing information on species variability, it also gave us a more accurate picture of cottid species distribution in the lake. Characters were selected on the basis of comparisons between our most homogeneous stocks. These were 534 sculpins from a 33- to 73-m depth in Lake Ontario and 95 sculpins from Bloomfield Creek. The four characters selected were as follows:

	C. bairdi	*C. cognatus*	Separation
Ratio: $\frac{\text{caudal peduncle length}}{\text{postorbital length}}$	<1	1	88%
Pelvic fin rays	4	3	97%
Last two anal rays	Same basal	Separate basal	95%
Palatine teeth	Present	Absent	82%

Other characters, like numbers of dorsal and anal rays, also gave good separation, but meristic features tend to be strongly correlated and an advantage of this analytical approach was that the characters were not so likely to be phenotypically or genotypically linked. An estimate of homogeneity of stocks was arrived at by assigning fish to either of the species on the basis of all four characters.

Discriminant Functions Analysis

In a further effort to maximize separation of taxa we combined the information in the entire character set in multivariate analyses. Discriminant function analysis is often used to identify specimens when species are very similar and cannot be reliably identified using single characters. Discriminant function analysis computes weights and adds the weighted characters into a single score so as to maximize separation between the groups. The analysis requires that the specimens be identified a priori on the basis of information not used in the analysis. In some cases we identified specimens to group using locality (stream versus lake *C. bairdi*). In other cases principal components analysis was used to cluster the specimens in two groups, each consisting of a single species. These two groups could then be exposed to discriminant function analysis to calculate weights for specimens of known identity. These weights were then used to compute the discriminant scores of specimens of unknown or doubtful identity.

Multivariate normality, continuous variables, and homoscadicity of sample dispersion matrices are assumed by most multivariate analyses. However, discriminant function and principal component analyses have been demonstrated to be quite robust to considerable deviations from these assumptions (Blackith and Reyment 1971; Sneath and Sokal 1973). In fact, both principal components analyses and discriminant function analyses of varying subsets of morphometric and

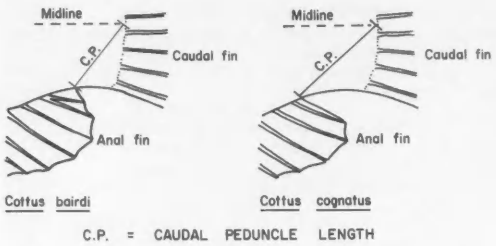

FIG. 2. Anal fin differences illustrate that in *C. bairdi* the last two rays are basally approximated (double) and in *C. cognatus* they arise from separate basals (single).

meristic characters, and different samples of specimens in our study yielded very similar results, and this confirms the views of these authors.

FORTRAN program 30 for principal components analysis and program 31 for discriminant analysis from Davies (1971), translated into BASIC, were used. All analyses were run on a Hewlett–Packard 9830 minicomputer system (McAllister et al. 1978) with 12-digit precision.

Data from a sample of 50 specimens from each stock were used in the multivariate analysis. Samples were as follows:

1) From Bloomfield Creek; 25 males, 25 females, 51–107 mm in length.

2) From a 9-m depth in Lake Ontario (Yorkshire Shoal); 22 males, 24 females, and 4 juveniles, 31–88 mm in length.

3) From a 40- to 55-m depth in Lake Ontario; 25 males, 25 females, 41–106 mm in length.

The following characters were used in the multivariate analyses. These characters were selected because they yielded separation values in excess of 80% for previously described populations (McAllister 1964), and because they did not involve overlap of meristics.

1) Log base 10 of caudal peduncle/log base 10 postorbital distance (log conversion done to reduce allometric influence).

2) Anal ray count.

3) Last two anal rays single, arising from separate basals, or double, arising from same basal.

4) Dorsal ray count.

5) Last two dorsal rays arising from same or separate basals.

6) Presence or absence of palatine teeth.

7) Log base 10 total length.

8) Left pelvic fin ratio. } Length of fourth ray/length of

9) Right pelvic fin ratio. } second ray.

All of the above analyses suggested that the best diagnostic character was the pelvic ray count (see Results). Specimens with intermediacy for this character often had a mixture of other characters and were thus difficult to assign to species. Intermediate pelvic ray counts involve a count of three rays on one fin and four on the other, or combinations involving three and a half, this notation signifying that the length of the fourth ray is less than two thirds the length of the longest ray (Scott and Crossman 1969). Because such atypical sculpins comprised 13.5% of all Lake Ontario collections, a separate multivariate analysis of such individuals was warranted.

Our second series of analyses was directed at finding how to best separate *C. bairdi* and *C. cognatus* so that the relative taxonomic position of problematical specimens could be determined. The Bloomfield Creek and the 40- to 55-m Lake Ontario samples of *C. bairdi* and *C. cognatus*, respectively, were tested for homogeneity by means of a principal components analysis. The results validated the reference samples. A discriminant function analysis was then run on the two samples and the weights from this analysis were used to compute scores for unknown or problematical specimens by summing the weighted characters for each specimen.

Inclusion of the pelvic character in discriminant functions analysis introduces a measure of circular reasoning in analysis of atypical specimens, as they were identified on the basis of pelvic ray counts in the first instance. The above analyses were therefore also conducted without inclusion of the pelvic character. For analysis we selected 219 problematical specimens 27–106 mm in length with intermediate pelvic counts. The sample was composed of 81 males, 119 females, and 14 juveniles and contained the following subsamples.

1) Seventy-six fish with three and a half/four pelvic ray counts.

2) Sixty-nine fish with three and a half/three pelvic ray counts.

3) Thirty-seven fish with three and a half/three and a half pelvic ray counts.

4) Thirty-three fish with three/four pelvic ray counts.

SUBSPECIES DISTINCTIONS

A feature of Lake Ontario sculpin populations which emerged from the above analyses was that populations of *C. bairdi* from the lake were not identical with populations of *C. bairdi* sampled elsewhere. Hubbs (1926) reported a separate subspecies (*C. bairdi kumlieni*, Hoy) in the Great Lakes proper compared with tributaries (*C. bairdi bairdi*, Girard). Hubbs and Lagler (1964) report *C. b. kumlieni* in the Finger Lakes and St. Lawrence River, a few inland lakes of Michigan and Lake Attawapiskat, James Bay drainage.

The chief diagnostic character used to distinguish subspecies is the distance between the tip of the snout to the anus measured backwards from the anus (Hubbs and Lagler 1964). In *C. b. bairdi* this distance extends to a point nearer the base than the end of the caudal fin; in *C. b. kumlieni* it is nearer the end than the base. We measured this character and examined creek and lake *C. bairdi* for characters which might be used in making subspecies distinctions.

BREEDING EXPERIMENTS

Breeding experiments were run 4 yr in succession, in an attempt to establish whether hybridization is possible between *C. bairdi* and *C. cognatus*. The results of these experiments were inconclusive and so details of this work are not reported here.

Results

ESTIMATES OF SEPARATION LEVELS BETWEEN SPECIES

Table 1 gives the results of the comparisons between the previously described sculpin populations from Ontario and

TABLE 2. *Cottus* species definition for Lake Ontario collections, determined by assigning fish to either *C. bairdi* or *C. cognatus* on the basis of four characters considered simultaneously.

Stock	Numbers of specimens by class			
	C. bairdi	Mixed characters	*C. cognatus*	% deviant
C. bairdi from McAllister (1964)	58	4	0	6.5
Cottus from Bloomfield Creek	79	16	0	16.5
Cottus from Lake Ontario:				
1–10 m Yorkshire Shoal	77	117	2	59.7
11–30 m intermediate zone	119	315	142	53.8
33–73 m deepwater zone	0	229	305	42.9
C. cognatus from McAllister (1964)	0	22	43	51.2

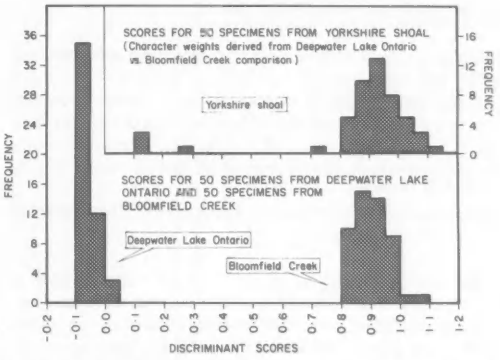
SCORES FOR 50 SPECIMENS FROM YORKSHIRE SHOAL
(Character weights derived from Deepwater Lake Ontario vs. Bloomfield Creek comparison)
Yorkshire shoal
SCORES FOR 50 SPECIMENS FROM DEEPWATER LAKE ONTARIO AND 50 SPECIMENS FROM BLOOMFIELD CREEK
Deepwater Lake Ontario
Bloomfield Creek
FREQUENCY
DISCRIMINANT SCORES

FIG. 3. Discriminant functions scores (pelvic ray character included) for samples of 50 sculpins from each of three locations: Bloomfield Creek, deepwater (40–55 m) Lake Ontario and Yorkshire Shoal, (9 m in depth) Lake Ontario.

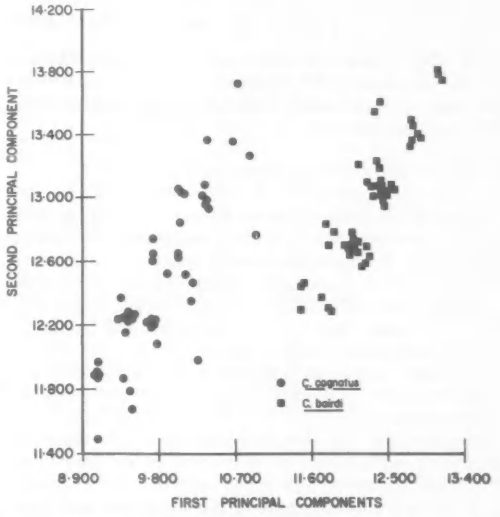

FIG. 4. Principal components analysis on 50 specimens from Bloomfield Creek and 50 specimens from 40 to 55 m in depth in Lake Ontario.

Quebec (McAllister 1964) with populations from Lake Ontario and Bloomfield Creek. As observed earlier, our populations gave poorer separation levels than those described by McAllister, and this was particularly the case for comparisons between populations within Lake Ontario. Unlike McAllister's stocks there was no diagnostic character which gave 100% separation in our materials. The caudal peduncle to postorbital ratio yielded only 66 and 81% separation levels for our two comparisons. Reduced effectiveness of this character was attributed to changes in population means rather than to an increase in variation. Parenthetically, this character was found to be highly correlated with sex; males of both species having relatively longer heads than females.

ESTIMATES OF VARIATION WITHIN DIFFERENT POPULATIONS

Table 2 shows the relative homogeneity levels determined on the basis of four characters. The Bloomfield Creek and Yorkshire Shoal collections were generally referable to *C. bairdi*, with few fish clearly referable to *C. cognatus*. The deepwater collections similarly contained no clear *C. bairdi* and at intermediate depths fish were referred to both species. Of greater interest here, however, are the differences in heterogeneity between the collections. Our diagnostically purest collections were from Bloomfield Creek, and the 16.5% contributed by variant fish is significantly ($P \sim 0.5$) higher than the 6.5% previously reported (McAllister 1964). The 1- to 10-m Lake Ontario collections from Yorkshire Shoal show the highest proportion of mixed characters, despite the fact that most of the "pure" types are referable to the less variable *C. bairdi*. The difference between these collections and McAllister's in incidence of mixed characters was highly significant ($P < 0.01$). The proportion of variant fish from collections in which *C. cognatus* predominates were also high, but because differences between the Lake Ontario and McAllister collections were not significant, we inferred therefore that this test simply confirmed the high degree of variability of this species across its range — a contention which is supported by the literature (see, for example, Van

TABLE 3. Principal component structure for 50 *Cottus* from Bloomfield Creek and 50 *Cottus* from a 40-m depth in Lake Ontario. Cumulative percentage of these four components is 93.3%.

Character	Principal component number			
	1	2	3	4
Caudal peduncle/postorbital ratio	−0.094	0.562	−0.075	0.111
Anal ray count	0.356	0.327	−0.090	−0.122
Last two anal rays single or double	0.302	0.419	−0.088	−0.026
Dorsal ray count	0.403	−0.166	−0.148	−0.395
Last two dorsal rays single or double	0.371	−0.142	−0.289	0.854
Palatine teeth	0.298	−0.043	0.921	0.170
Total length	0.159	0.542	0.100	−0.012
Left pelvic fin ratio	0.424	−0.167	−0.068	−0.181
Right pelvic fin ratio	0.424	−0.174	−0.100	−0.159
λ	4.853	2.592	0.650	0.306
%	53.925	28.804	7.218	3.395

Vliet 1964). Values obtained for the 11- to 30-m Lake Ontario collections, which we have designated the intermediate zone, are what one would expect from a mixture of shallow-water and deepwater stocks.

Discriminant Functions Analysis

Discriminant functions scores for our samples from deep and shallow-water Lake Ontario and for Bloomfield Creek are given in Fig. 3. All sculpins from the deep water sample were referable to *C. cognatus* and all creek fish were referable to *C. bairdi*. The bulk of Yorkshire Shoal fish were referable to *C. bairdi*; those which were not were those with pelvic ray counts intermediate between 3 and 4.

In theory, discriminant functions scores should serve as a better distinction between taxa than any one character taken singly (Sneath and Sokal 1973). As noted earlier, pelvic ray counts gave the best separation levels of all the characters examined, and thus received considerable weight. An average *C. bairdi* specimen with a ray count of three gave a *C. cognatus* score and vice versa. Because of this, specimens with intermediate pelvic counts were analyzed separately.

The first step in this analysis was the study of heterogeneity in the reference samples. The Bloomfield Creek and the 40- to 55-m Lake Ontario samples were tested by principal components analysis and produced the results shown in Fig. 4. The parameters used in the components analysis are shown in Table 3. Each sample clustered tightly, with no creek specimens falling in the lake cluster and no lake specimens falling in the creek cluster.

Discriminant functions analysis on the reference samples without inclusion of the pelvic character gave results (Fig. 5), which were essentially the same as when the pelvic character was included, although the separation was not as complete. Specimens with intermediate pelvic counts were extremely variable, with scores which spanned the scale (Fig. 5). Intermediates with low counts (three/three and a half) tend more towards scores on the *C. cognatus* side than specimens with higher counts, which tend more towards *C. bairdi* scores. An analysis of the variance in the discriminant functions scores gave the results in Table 4, and confirmed that intermediate types are far more variable than "pure" types, particularly those with counts of three/four and three and a half/three and a half pelvic rays.

Running the analysis without pelvic characters increased the weight on the proximity of last two rays of anal fin character. This character was highly correlated with pelvic counts and with depth, as can be seen in the bottom line in Fig. 6.

Number and Distribution of Intermediate Specimens in Lake Ontario

Intermediate pelvic ray counts were far more common in Lake Ontario than counts of less than three or more than four (13.5% compared with 0.1%). The problem of specimens with intermediate pelvic counts is best demonstrated by Fig. 6, which illustrates the following important points.

1) Pelvic ray count frequencies change continuously with depth, but with a particularly pronounced area of change between 30 and 33 m.

2) Intermediate specimens comprise a substantial proportion (22%) of the catch from 9 to 26 m, their numbers diminishing above and below these depths. We would likely find intermediate specimens in collections from all depths, with more extensive sampling. Note that the two apparently "pure" collections comprised only 33 (1−6 m) and 43 fish (43 m).

3) The number of intermediates on Yorkshire Shoal did not differ significantly from those of the intermediate zone (11−30 m) and both collections had significantly higher numbers of intermediates than did deepwater zone collections. Figure 6 indicates gradation of Yorkshire Shoal into the intermediate zone; particularly in terms of increasing numbers of *C. cognatus* with increasing depths.

Our data and those of other workers (Wells 1968; Semple 1977) suggest that *C. cognatus* may be found in shallow water during the colder months, but that they move to cooler depths during the summer. We also found *C. bairdi* at greater depths during the summer months than at other times of the year; the relatively high percentage frequency of four-rayed fish at 20−30 m reflects the fact that these samples were collected in July.

The percentage frequencies of specimens with damaged pelvic fins (see bottom of Fig. 6) created a further diagnostic problem. Damaged fins were common (7%) in collections

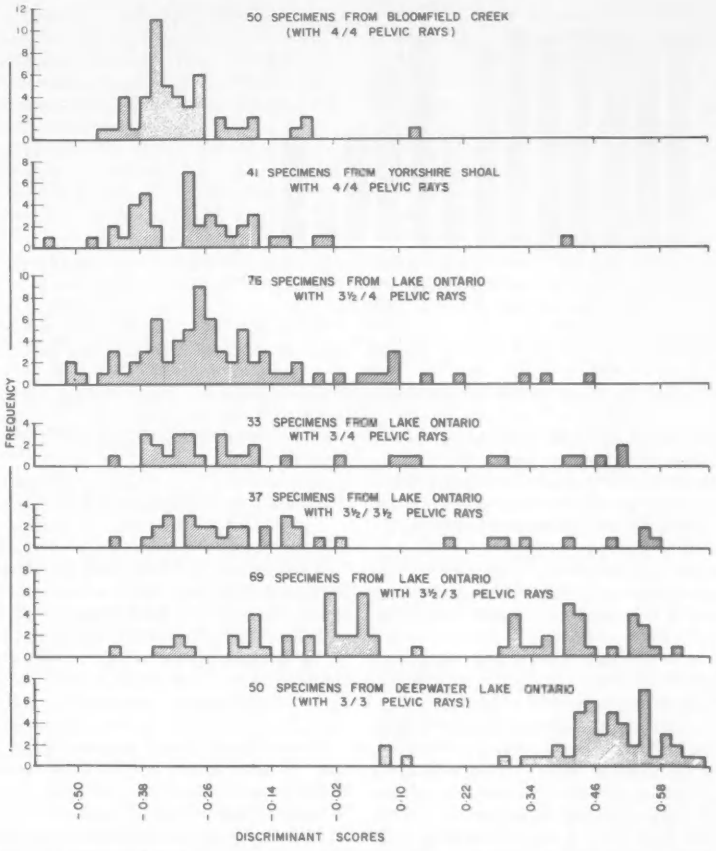
50 SPECIMENS FROM BLOOMFIELD CREEK (WITH 4/4 PELVIC RAYS)
41 SPECIMENS FROM YORKSHIRE SHOAL WITH 4/4 PELVIC RAYS
76 SPECIMENS FROM LAKE ONTARIO WITH 3½/4 PELVIC RAYS
33 SPECIMENS FROM LAKE ONTARIO WITH 3/4 PELVIC RAYS
37 SPECIMENS FROM LAKE ONTARIO WITH 3½/3½ PELVIC RAYS
69 SPECIMENS FROM LAKE ONTARIO WITH 3½/3 PELVIC RAYS
50 SPECIMENS FROM DEEPWATER LAKE ONTARIO (WITH 3/3 PELVIC RAYS)
FREQUENCY
-0.50
-0.38
-0.26
-0.14
-0.02
0.10
0.22
0.34
0.46
0.58
DISCRIMINANT SCORES

FIG. 5. Discriminant functions scores run without the pelvic ray character. Samples from Bloomfield Creek and deepwater Lake Ontario were the same as those in Fig. 3. The Yorkshire Shoal sample was the same as in Fig. 3, except that nine anomalous specimens with intermediate pelvic ray counts were not included here, but below, in the samples of specimens with intermediate pelvic ray counts. Intermediate specimens from Lake Ontario were analyzed separately in four subsamples, determined by the pelvic ray count.

from 9 to 30 m, and particularly with those from 9 to 10 m (21%), but did not occur in our shallowest collections from 1 to 8 m. We are unable to account for this.

SUBSPECIES ANALYSIS

Estimates of separation levels (Table 1) indicated that Lake Ontario *C. bairdi* were less distinct from Lake Ontario *C. cognatus* than were Bloomfield Creek *C. bairdi*. Estimates of homogeneity of stocks from the four-character analysis (Table 2) indicated that lake *C. bairdi* were less homogeneous than creek *C. bairdi*. Discriminant functions analysis showed that over 90% of Bloomfield Creek *C. bairdi* could be distinguished from Lake Ontario *C. bairdi* (the 9-m sample), confirming that there are significant differences between these

TABLE 4. Discriminant functions scores aggregated by pelvic fin ray counts.

Stock	Pelvic rays	Mean	Coefficient of variation, %
Bloomfield Creek	4/4	−0.299	35.2
Yorkshire Shoal	4/4	−0.264	59.3
Intermediates	3½/4	−0.212	91.7
	3/4	−0.048	615.2
	3½/3½	−0.072	415.0
	3/3½	0.143	209.6
Deepwater zone	3/3	0.466	26.8

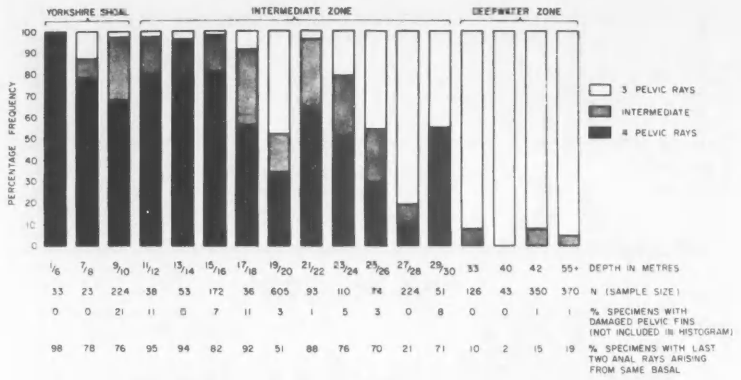
YORKSHIRE SHOAL
INTERMEDIATE ZONE
DEEPWATER ZONE
PERCENTAGE FREQUENCY
100
90
80
70
60
50
40
30
20
10
0
3 PELVIC RAYS
INTERMEDIATE
4 PELVIC RAYS
1/6 7/8 9/10 11/12 13/14 15/16 17/18 19/20 21/22 23/24 25/26 27/28 29/30 33 40 42 55+ DEPTH IN METRES
33 23 224 38 53 172 36 605 93 110 74 224 51 126 43 350 370 N (SAMPLE SIZE)
0 0 21 11 0 7 11 3 1 5 3 0 8 0 0 1 1 % SPECIMENS WITH DAMAGED PELVIC FINS (NOT INCLUDED IN HISTOGRAM)
98 78 76 95 94 82 92 51 88 76 70 21 71 10 2 15 19 % SPECIMENS WITH LAST TWO ANAL RAYS ARISING FROM SAME BASAL

FIG. 6. Correlation between depth and pelvic ray counts in Lake Ontario sculpins.

stocks. According to Sneath and Sokal (1973) such differences would probably be at the subspecies level. Bailey et al. (1954) recommend a level of 93%, with high uniformity of each subspecies over its range and intergrading only over a narrow geographical area. Further study is needed to substantiate whether or not these conditions are met in *C. bairdi*.

Using the subspecies diagnostic character of snout-to-anus measurement (see Materials and Methods) of Hubbs and Lagler (1964), we obtained a separation of only 58% when comparing Yorkshire Shoal *C. bairdi* with creek *C. bairdi*, though differences between stocks were highly significant ($P < 0.001$). There is therefore some basis for separation but insufficient grounds for modern subspecies recognition. Other distinguishing characters noted by Hubbs and Lagler (1964) were less useful. They noted, as we did, that *C. b. kumlieni* had less distinct mottling and a more slender body type. Pigmentation differences are difficult to quantify, and the slender body form of this morph could be a by-product of the method of capture, because most *C. bairdi* specimens were caught in traps and were usually starved. There were significant differences (*t*-tests) between length–weight regression slopes of *C. cognatus* that were trapped and *C. cognatus* that had been captured in trawls.

We found that *C. bairdi* from the lake were smaller than *C. bairdi* from the creek. Creek *C. bairdi* averaged 73 mm total length, attained a maximum size of 107 mm, and the smallest mature female was 61 mm; lake *C. bairdi* averaged 56 mm, attained a maximum size of 88 mm, and the smallest mature female was 45 mm. Semple (1977) reported a maximum size of 81.5 mm standard length (~ 97 mm total length) for lake *C. bairdi* and Koster (1936) gave the maximum size-class for both *C. bairdi bairdi* and *C. bairdi kumlieni* as 65–70 mm standard length (~ 77–83 mm total length). Thus the evidence for size differences is inconclusive and may be due to environmental effects.

Because length measurements were included in the discriminant functions analysis, we cannot exclude the possibility that part of the discrimination found in this analysis between *C. bairdi* populations was due to size differences in the population. In summary, subspecies distinctions are presently unclear and cannot be verified until the diagnostic characters of the subspecies are examined and comparisons made between lakes.

ASSESSING THE POTENTIAL FOR HYBRIDIZATION

Data from our breeding experiments confirmed the observation of Koster (1936) that there are differences in preferred breeding temperatures and times for the same three cottid stocks. Our field data also supported these observations. Lake Ontario *C. cognatus* spawns slightly earlier (May 11–25) and at slightly colder temperatures (4–6°C) than lake *C. bairdi* (May 18–June 13 at 5–8°C). In 1977, Bloomfield Creek *C. bairdi* males were found attending nests with eggs which were laid May 6–10, at water temperatures of 9–13.5°C.

Gravid fish of "pure" and intermediate types have been taken at various depths in Lake Ontario and most particularly in the intermediate zone. Field data which suggested that hybridization may be possible came from traps set May 8 and lifted May 18, 1979. The traps were set at 22- and 27-m depths on rock bottom. They contained both ripe and spent females of both *C. cognatus* and *C. bairdi*. Bottom temperatures were 5°C when set and also when lifted.

Ripe and spent females of *C. bairdi* were caught from the shoreline to 27 m during the spawning season. Ripe and spent females of *C. cognatus* were caught during the spawning season from 17 m to the maximum depth sampled at this time, which was 55 m. Thirty-seven percent *C. bairdi* spawning grounds by depth are in contact with no more than 26% of *C. cognatus* spawning grounds by depth as *C. cognatus* grounds probably extend below the maximum depth sampled. We inferred, therefore, that there is a greater likelihood of *C. bairdi* making contact with *C. cognatus*, than the reverse.

Summary and Discussion

Our results indicate that the decreased ability to identify the species of *Cottus* in Lake Ontario arises from two sources: (1) the greater variability of *C. bairdi* in the lake compared to the surrounding drainage area and (2) significant numbers of intermediate specimens. Analysis of diagnostic characters

established that the best means of distinguishing *Cottus* species in Lake Ontario was the pelvic ray count; four pelvic rays in *C. bairdi* (with the fourth ray at least two thirds the length of the longest ray) and three pelvic rays in *C. cognatus*. The next best character was the condition of the last two rays of the anal fin; arising from the same basal in *C. bairdi* or separate basals in *C. cognatus*. We suggest that these characters be used in conjunction for distinguishing Lake Ontario *Cottus*. We further suggest that the 13.5% of Lake Ontario specimens with intermediacy in the pelvic ray character be provisionally designated hybrids, at least until further clarification of the identity of such intermediates becomes possible. The discussion which follows develops our argument for acceptance of this hypothesis.

First, we could find no basis from which to relate phenotypic variability to relative environmental rigorousness. The sampling depths below about 10 m would presumably not differ sufficiently in terms of day-to-day changes in temperature, oxygen, etc., to explain the observed variations which we infer must have their origins in the early life history phases.

Hybrid fishes are usually identified on the basis of intermediacy of the suspected hybrid specimens in a number of characteristics that serve to discriminate the parental species (Hubbs 1955). However, there are a number of other phenomena which lead to conditions of intermediacy, which might be confused with hybridization. Such phenomena include (1) character convergence, (2) the development of morph-ratio step-clines (Clarke 1966; Endler 1973), and (3) the spread of newly evolved characteristics through the range of a species (Eldredge and Gould 1972). Schueler and Rising (1977) discuss the problem of separating hybridization from "hybridization-like" phenomena in the all-too-common situation where only phenetic evidence is available. They conclude that "an increase in variability and intermediacy in concert constitute the only phenetic evidence of hybridization." Thus a failure to find an increase in variance does not negate the possibility that hybridization is taking place, but increased variance does support a hypothesis of hybridization.

If intermediacy in the pelvic character represents the hybrid condition, we would expect such individuals to have intermediate discriminant functions scores (Greenfield and Greenfield 1972), and this was found to be the case. An examination of the variance around discriminant functions scores (Table 4) makes it apparent that intermediates are more variable than normal fish. The criteria of Schueler and Rising (1977) thus support the hybridization hypothesis.

Hubbs (1955) noted that hybridization is more likely where there is a disparity in the population size between the two parent species. The relatively restricted distribution of *C. bairdi* on the periphery of the lake, together with data from catch records, suggest that *C. bairdi* is less numerous than *C. cognatus*.

If it is accepted that hybridization accounts for the presence of sculpins intermediate in appearance between *C. bairdi* and *C. cognatus*, we must also account for the higher variance of *C. bairdi* in the lake compared with creek *C. bairdi*. The suggestion that lake *C. bairdi* is the more variable *C. bairdi kumlieni* subspecies described by Hubbs and Lagler (1964) was discussed earlier. Another possibility is that the increase in variation might be ascribed to backcrossing. If backcrossing is occurring we would expect its greatest effect in the smaller of the two parent populations. This could be especially so in this case where the smaller population is surrounded by the hybrid zone. It may be significant that the *kumlieni* subspecies is reported only from the Great Lakes and other large bodies of water where the disparity of parent population sizes would be particularly conducive to hybridization, and where we might expect swamping of the smaller *C. bairdi* population by backcrossing.

Several features of the discriminant functions analysis offer support for the backcrossing hypothesis. These are (1) the lack of a prominent central peak in the frequency of discriminant functions scores, (2) the high variance of putative hybrids, and (3) skewing of the parent stocks toward the center. This latter feature is one of the means proposed by McAllister et al. (1972) and Butcher (1980) of recognizing introgressive hybridization. Other means suggested by Butcher (1980) are a progressive increase in hybrid frequency or an increasing variance of parental distributions relative to the control distribution. Our data are suggestive in this regard, but confirmation cannot be obtained without studying changes in the local population over an extended period of time.

If introgressive hybridization is taking place it could lead eventually to a complete swamping of species. That this has not taken place may be attributable to a recent onset of hybridization. Recent biotic and abiotic changes in the Lake Ontario environment have been extensive (Thomas and Christie 1982). Such changes could have induced hybridization by altering species balance. Hubbs (1955) stressed the importance of environmental factors in inducing hybridization in fish. One such change which may have affected Lake Ontario sculpins was the relaxation in predation pressure resulting from the collapse of lake trout populations which occurred in the 1940s (Christie 1972). However, evidence from Hubbs and Lagler (1949) from collections made in 1945 indicates that species distinction in Great Lakes sculpins was problematical even prior to the lake trout collapse. It may be, therefore, that new collections and methodology may be revealing a phenomenon of long standing.

If introgressive hybridization has been taking place between *C. bairdi* and *C. cognatus* over an extended period of time, species integrity may have been maintained by the presence of a partial barrier such as reduced fertility of hybrids or slight differences in spawning requirements. Differences in preferred spawning times and temperatures do appear to exist. Differences in the range of spawning grounds are also implicated because many ripe *C. cognatus* were caught outside the range of *C. bairdi* during the spawning season.

Hybridization studies are complicated when the parent species are similar or variable, hybrids are numerous, or backcrossing is occurring (Smith 1973). Many, if not all, of these difficulties have been encountered in this study and have complicated the interpretation of results. Full confirmation of the hybridization hypothesis will require an alternate approach, such as that provided by electrophoresis.

Acknowledgments

We thank P. E. Ihssen for helpful criticism of the manuscript and

for advice on data interpretation. E. J. Crossman provided valuable background information and advice on identification of sculpins. We thank the staff at the Glenora Fisheries Station who were involved in the project. W. F. Miller maintained sculpins over winter for breeding experiments in addition to assisting with field collections. M. T. Walters and S. M. LeGrow contributed importantly to the taxonomic routines, and S. Leonard prepared the figures. The project was funded by the Ontario Ministry of Natural Resources.

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An Evaluation of the Wet Oxidation Technique for Use in Determining the Energy Content of Seston Samples[1,2]

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Many of the techniques available to determine energy content have problems inherent in their use on samples with a high proportion of inorganic minerals. One technique which may readily be used on such samples is wet oxidation, in which the sample is quantitatively chemically oxidized. However, this technique is infrequently used because of a potentially significant underestimation of energy content, due to incomplete oxidation of nitrogenous compounds. For a number of plant and animal tissues, wet oxidation with potassium dichromate gave values for energy content within ±3.0% of the value determined by bomb calorimetry.

Seston samples on glass fiber filters may also be quickly and accurately analyzed using this procedure. The conversion from either weight loss on ignition or carbon and nitrogen content to energy content, using literature-derived conversion factors, is extremely inaccurate. Conversion of the wet oxidation values to carbon content gave inconsistent results. If the carbon content of the samples is required this should be determined directly by C:N analysis, or by quantifying the CO_2 liberated by wet oxidation.

Key words: wet oxidation, seston analysis, energy budgets, calorimetry, weight loss on ignition, CHN analysis

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Parmi les méthodes de détermination du contenu énergétique, il en est plusieurs qui, lorsque utilisées sur des échantillons à forte proportion de minéraux inorganiques, ont des problèmes inhérents. Sur de tels échantillons, une méthode facilement utilisable est l'oxydation humide, selon laquelle l'échantillon est oxydé quantitativement par des moyens chimiques. Cependant, on ne l'utilise pas souvent parce que, à la suite d'une oxydation incomplète des composés azotés, elle peut sous-estimer de façon significative le contenu énergétique. Pour plusieurs tissus végétaux et animaux, l'oxydation humide avec bichromate de potassium donne un contenu énergétique qui s'écarte de ±3,0 % de celui déterminé par bombe calorimétrique.

Cette méthode permet également d'analyser rapidement et précisément des échantillons de seston sur filtres de fibres de verre. La conversion en contenu énergétique à partir, soit de la perte de poids à la combustion, soit de la teneur en carbone et azote, en utilisant des facteurs de conversion extraits de la littérature, est extrêmement imprécise. La conversion en teneur en carbone des valeurs d'oxydation humide donne des résultats contradictoires. Si l'on doit connaître la teneur en carbone des échantillons, il faudra la déterminer directement par analyse de C:N, ou par quantification du CO_2 libéré par l'oxydation humide.

Received December 16, 1981
Accepted June 15, 1982

Reçu le 16 décembre 1981
Accepté le 15 juin 1982

MANY studies of energy flow in marine ecosystems require an accurate means of determining the energy and nutrients available to the successive stages of the food web. The most direct method for estimating the actual energy content of food materials is by bomb calorimetry. However, this method may only be applied to samples with a low inorganic content, due to possible errors associated with endothermic reactions. Also,

[1]Contribution No. 1325 from the Center for Environmental and Estuarine Studies, University of Maryland.

[2]This work was partially supported by a N.A.T.O. postdoctoral fellowship and a Maryland sea grant (RF/14) to the author and an NSF Grant DEB 7908862 and U.S. Public Health Service Grant GM21133 to Dr R. K. Koehn.

Printed in Canada (J6756)
Imprimé au Canada (J6756)

dried seston and sediment samples do not burn well and the addition of large amounts of benzoic acid, to aid combustion, can lead to considerable inaccuracies in the calculation of energy content (Crisp 1971).

A frequently used and rapid method of estimating the organic content of seston samples is to determine the weight loss upon ignition. This technique determines the total mass of particulate organic matter (P.O.M.) but is subject to several errors. The weight change may include losses associated with heat-mediated changes in the inorganic component of the sample. Also, as it is an indirect proportion measurement, its accuracy is inversely related to the amount of organic material present.

Quantitative biochemical analysis of protein, lipid, and carbohydrate has been used to determine nutrient availability in estuarine water (Widdows et al. 1979), but this requires three relatively complex analyses per sample. The carbon, hydrogen, and nitrogen content of biological samples may be accurately determined using instrumental elemental analysis, but an empirically determined factor for each set of samples must be used to convert these values to energy content.

Quantitative chemical oxidation enables both the energy and carbon content of organic samples to be determined. In its simplest form, this technique involves oxidizing the organic molecules to their basic elements using a strong oxidizing agent (e.g. potassium permanganate or potassium dichromate) and quantitatively determining how much oxidant has been reduced (Walkley and Black 1934; El Wakeel and Riley 1957; Maciolek 1962). This method has been extensively used to determine energy content, by converting the "oxygen demand" of the organics to energy values using a mean oxycalorific value of 14.14 $J \cdot mg^{-1}$ O_2 calculated from proteins, lipids, and carbohydrates (Elliot and Davison 1975). Although this conversion is only absolutely accurate if the composition of the sample is known, in practice there would only be a maximum of 4% error introduced even if the sample was entirely carbohydrate.

The chemical oxidation technique is not in wide use today for estimating the energy and nutrient content of natural samples, despite its ease of application, because of widespread criticism of its ability to oxidize fully organics rich in nitrogen (Hughes 1969; Crisp 1971). However, a detailed description and evaluation of a wet chemical oxidation procedure (Maciolek 1962) indicated a high degree of oxidation (mean energy content within 5.5% of the value) for purified compounds rich in nitrogen, e.g. gelatin, uric acid, and cystine. The aim of the research described in this paper was to test the efficiency of the wet oxidation procedure on samples of plant and animal origin. These materials were simultaneously analyzed for energy content by bomb calorimetry, for particulate organic matter by weight loss on ignition, and for carbon and nitrogen content by instrumental C:N analysis.

Materials and Methods

A pellet of the unicellular alga, *Tetraselmis suecica* (Kylin) Butch, was obtained by centrifugation of a monoculture. The pellet was then washed twice with distilled water. Filamentous algae, *Enteromorpha* sp., was freshly collected from the intertidal zone at Stony Brook, NY and rinsed of epibionts with distilled water. Senescent stems of the terrestrial angiosperm, *Phragmites australis*, were rinsed in distilled water and macerated in a blender. Gonadal and somatic tissue of 25 mussels, *Mytilus edulis*, was collected in November and April from two populations on Long Island, NY. All samples were oven-dried to constant weight at 95°C before being finely ground in a Wiley mill. Seston samples were collected from Stony Brook, NY, by vacuum-filtering volumes of seawater through Whatman GF/C filters (2.4 cm in diameter) until the seston clogged the filter. Chloride ions were removed from the seston samples by rapidly sucking 4 mL of isotonic ammonium formate through the filter. The isotonic ammonium formate prevents cells lysing, with the consequent loss of intracellular organic compound, but sublimes when the filters are dried at 95°C, prior to weighing. Filters which had only ammonium formate passed through were also processed and used throughout as blanks.

Between 0.8 and 1.0 g of each of the organic materials was compressed into a pellet and accurately weighed. This pellet was then combusted in a Parr adiabatic bomb calorimeter, using normal operating procedure. This was performed in triplicate for each substance, except the animal tissues, which were duplicated. The energy content was calculated using a calibration factor determined using standard benzoic acid.

The carbon and nitrogen analyses were performed on a Hewlett–Packard CHN analyzer, using standard operating procedures. The instrument was frequently calibrated against acetanilide.

Samples of the materials were weighed in tared crucibles and then ashed for 4 h at 450°C in a muffle furnace. The crucibles were cooled in a desiccator and reweighed. The weight loss on ignition (P.O.M.) was then determined by difference.

The wet oxidation procedure used in this study was originally described by Walkley and Black (1934) and modified by Maciolek (1962).

A known weight of test material (about 10.0 mg) or a filter, carefully curled using forceps so that the coated surface was inward, was placed in a 25-mL Pyrex tube (the weight required is dependent on the proportion of organics present and the volume of reagents used, but sufficient organic material must be added so as to reduce about 20% of the dichromate). Exactly 3.0 mL of the 2 *N* potassium dichromate solution (98.6 g of $K_2Cr_2O_7$ oven-dried at 150°C for 3 h, made up to 1 L) was pipetted into each sample tube and into two control tubes, one without organics and one with a blank filter. About 6.0 mL of concentrated sulfuric acid (sp gr 1.82), to which silver sulfate had been added to give a 1.2% W:V solution, was rapidly added directly to the surface of the dichromate solution, using a long syringe. The tubes were agitated for 60 s, capped with a glass marble, and half immersed for 3 h in a boiling water bath during which time they were frequently agitated. On the last agitation, the tubes were held on a shallow angle and rotated such that the lower $\frac{1}{4}$ of the tubes were rinsed with the oxidizing mixture. After cooling, the mixture was transferred to a conical flask and the tube (with filter) rinsed with 150 mL of distilled water. Five millilitres of concentrated phosphoric acid (85%) and ~0.5 mL of a 0.3 W:V solution of barium diphenylaminesulfonate indicator were ad-

TABLE 1. Energy content of three plants and the germinal and somatic tissue from the mussel, *Mytilus edulis*, estimated by bomb calorimetry, wet oxidation, and weight loss on ignition at 450°C. When the number of replicates (n) > 2, the mean ± SD is presented. The P.O.M. values were converted to Joules using a factor derived from the literature of 20.1 KJ·g^{-1} P.O.M. (See text for details.) None of the ANOVA F values were significant at $P > 0.05$.

	1	2	Weight loss on ignition			
Material	Bomb calorimetry, J·g^{-1} dry wt	Wet oxidation, J·g^{-1} dry wt	% difference between 2 and 1	ANOVA[a] F value between 1 and 2	P.O.M., mg·g^{-1} dry wt	Energy content, J·g^{-1} dry wt
Tetraselmis suecica	19674.6±403.8 n = 3	19557.6±309.2 n = 13 On filter 18984.1±381.9 n = 4	−0.6% −3.5%	0.319 5.35	808.42±38.9 n = 5	16249.0
Enteromorpha sp.	18619.5±244.2 n = 3	18022.9±841.1 n = 6	−3.2%	0.157	935.9±15.3 n = 3	18811.0
Phragmites australis	18824.8±270.3 n = 3	18913.2±1010.6 n = 17	+0.5%	0.022	967.2±3.5 n = 6	19440.0
Mussel Somatic (Nov. 1979) (Stony Brook)	22105.2 22219.9 n = 2	21440.6±133.7 n = 3	−3.3%	—	868.3±19.1 n = 4	17453.0
Mussel Germinal (April 1980) (Shinnecock)	19392.1 19408.5 n = 2	18233.6±428.2 n = 3	−6.0%	—	836.9±11.6 n = 4	16822.0
Mussel Somatic (April 1980) (Stony Brook)	22621.4 22355.7 n = 2	21290.1±494.0 n = 3	−5.3%	—	ND[b]	—

[a] Analysis of variance (Sokal and Rohlf 1969).
[b] ND, no data.

ded to the solution. A ferrous sulfate solution ~0.75 N (made by dissolving 210 g of ferrous sulfate in 980 mL deionized water and 20 mL of concentrated H_2SO_4 and stored in the dark at 4°C until used) was then titrated against the remaining dichromate. The oxidizing mixture is a dark greenish/blue on the addition of the indicator and a few drops of ferrous sulfate, but gradually becomes a light green/mauve, prior to turning bright green at the end point.

The normality of the ferrous sulfate (N $FeSO_4$) was determined by titrating it against the 2 N potassium dichromate (Eq. 1).

$$(1)\quad \text{N } FeSO_4 = \frac{\text{Vol. dichromate (mL)} \times \text{normality of dichromate}}{\text{Vol. } FeSO_4 \text{ (mL)}}$$

The weight of oxygen (O.C mg) required for complete oxidation of the organics was calculated using Eq. (2).

$$(2)\quad \text{O.C.} = (A - B) \times \text{N} \times 8$$

(with all volumes in millilitres) where A = volume of ferrous sulfate required for titration of the blank, B = volume of ferrous sulfate required for titration of the sample, N = normality of the ferrous sulfate, and 8 is the equivalent weight for oxygen.

The oxygen required (O.C.) may then be converted to energy content using a conversion factor of 14.14 J·mg^{-1} O_2 (Elliot and Davison 1975). It may also be converted to carbon content using a conversion factor derived by Maciolek (1962) of carbon content = O.C./2.86.

Results

The results of the various analyses of the homogenous organic materials are listed in Tables 1 and 2.

The energy content of the unicellular alga *T. suecica* (19.7 KJ·g^{-1} dry wt) is lower than the value of 23.4 KJ·g^{-1} dry wt determined by Widdows and Bayne (1971) for this species, but this may be due to different culture conditions. However, the energy content of 18.62 KJ·g^{-1} dry wt for *Entermorpha* sp. is slightly higher than that recorded for three species of Pacific Coast seaweed (range 11.3–15.5 KJ·g^{-1} dry wt) by Himmelman and Carefoot (1975) but similar to the range of 17.5–20.4 KJ·g^{-1} dry wt for green algae quoted by Kenchington (1970). The slight discrepancy may be due to differences between species of *Enteromorpha* or simply be a reflection of seasonal variation in energy content. The value for the energy content of *P. australis* (18.8 KJ·g^{-1} dry wt) is almost identical with that reported for the species (18.9 KJ·g^{-1}) by Cahoon and Stevenson (unpublished data). The

TABLE 2. Carbon content of three plants and the germinal and somatic tissue from the mussel, *Mytilus edulis*, estimated by wet oxidation and instrumental C:N analysis. All values are presented as the mean of *n* observations ± SD. (Significance levels for ANOVA, $*P > 0.05$, $***P > 0.001$.)

	Instrumental C:N analysis,				
	1	2			
Material	Wet oxidation, mg C·g^{-1} dry wt	Carbon mg·g^{-1} dry wt ± SD	Nitrogen mg·g^{-1} dry wt ± SD	C:N ratio	ANOVA[a] *F* value between (1 and 2)
Tetraselmis suecica	483.2±7.6 *n* = 13 On filter 474.9±12.1 *n* = 4	371.4±37.8 *n* = 6	45.4±8.5 *n* = 6	8.2	111.68***
Enteromorpha sp.	443.9±18.9 *n* = 6	ND[b]	ND[b]	—	—
Phragmites australis	467.3±24.2 *n* = 17	476.9±7.9 *n* = 7	7.6±2.2 *n* = 7	62.8	0.982
Somatic (Nov. 1979) (Stony Brook)	529.7±3.3 *n* = 3	479.7±63.5 *n* = 4	70.2±11.7 *n* = 4	6.8	1.74
Germinal (April 1980) (Shinnecock)	450.5±10.6 *n* = 3	424.9±28.1 *n* = 4	71.2±2.2 *n* = 4	6.0	2.12
Somatic (April 1980) (Stony Brook)	526.0±12.2 *n* = 3	480.9±26.9 *n* = 5	101.3±3.9 *n* = 5	4.7	7.11*

[a] Analysis of variance (Sokal and Rohlf 1969).
[b] ND = no data.

tissue energy content of the mussel, *M. edulis*, ranged between 19.39 and 22.62 KJ per gram dry body weight which is similar to the mean value of 20.6 KJ per gram dry body weight recorded for the species by Dare and Edward (1975).

The energy content values obtained from the wet oxidation analyses were all slightly lower (except for *P. australis*) than those obtained by bomb calorimetry. A one-way analysis of variance (ANOVA) (Sokal and Rohlf 1969) for the data sets with more than two replicates indicated that there were no significant differences between the values determined using the two methods. The results of the wet oxidation analysis of the *T. suecica* samples on Whatman GF/C filters indicated that there was no significant interference of the filter on the determination of energy content. The relatively large standard deviation for the wet oxidation values of *P. australis* is probably due to heterogeneity between the samples. Despite the fact that it was finely milled, it still had distinguishable pieces of stem and leaf. Consequently, it was difficult to obtain truly homogeneous 10-mg samples for testing.

A one-way ANOVA indicated that the value for carbon content estimated from instrumental C:N analysis and wet oxidation was not significantly different for *P. australis* and two of the animal tissue samples. However, the carbon content, as estimated from the wet oxidation results for *T. suecica* and one somatic animal tissue sample, was significantly higher (Table 2). The wet oxidation estimates for the carbon content of the seston samples were higher for three of the four samples than the values determined using instrumental C:N analysis (Table 3).

Platt and Irwin (1973) determined the relationship between energy and carbon and nitrogen content for a mixed bloom of phytoplankton as:

$$\text{Cal mg dry wt}^{-1} = -0.555 + 0.113\,(\%C) + 0.054\,(C{:}N)$$

When this relationship is applied to the *T. suecica* and *P. australis* data, values of 17.1 KJ·g^{-1} dry weight and 34.5 KJ·g^{-1} dry weight are obtained, respectively, which are 13% less and 83% more than those estimated from bomb calorimetry.

The P.O.M. value is obviously related to the energy content of the sample and if the appropriate conversion factor is known, it may be directly converted to energy content. Platt and Irwin (1973) determined the energy content of mixed phytoplankton samples and a conversion factor of 20.74 KJ·g^{-1} P.O.M. may be calculated from their Table 2. Jørgensen (1966) gave a similar value of 19.3–20.5 KJ·g^{-1} P.O.M. for phytoplankton. Ostapenya et al. (1967) (cited in Kenchington 1970) determined a linear relationship between energy content and P.O.M., expressed as Calorific Value = 4.91 × P.O.M. + 0.327. Bayne and Widdows (1978) considered 10.18 KJ·g^{-1} P.O.M., estimated from biochemical analysis, to be a reasonable conversion factor for their estu-

TABLE 3. Energy and carbon content of seston samples, from Stony Brook, New York, filtered onto Whatman GF/C filters, determined using the wet oxidation technique, instrumental C:N analysis, and weight loss on ignition at 450°C. All results are the mean of two replicates and are expressed per gram dry weight of total seston. The P.O.M. values were converted to Joules using a factor of 10.18 KJ·g^{-1} P.O.M. (Bayne and Widdows 1978).

	Wet oxidation		Instrumental C and N analysis			Weight loss on ignition	
Date	Energy content, J·g^{-1} seston	Carbon content mg C·g^{-1} seston	Carbon content, mg C·g^{-1} seston	Nitrogen content, mg N·g^{-1} seston	C:N Ratio	P.O.M., mg·g^{-1} seston	Energy content, J·g^{-1} seston
Aug. 9, 1978	11945.7	294.9	135.72	21.75	6.24	475.8	4843.6
Dec. 5, 1978	5395.9	133.2	61.55	7.79	7.91	191.2	1946.4
April 15, 1979	6074.2	149.9	175.49	25.06	7.0	326.0	3318.7
June 12, 1979	8476.4	209.3	142.15	18.77	7.57	393.0	4000.7

arine seston samples, which were rich in detritus.

For the purpose of this study, two P.O.M. to energy content conversion factors were used: one of 20.1 KJ·g^{-1} P.O.M. (mean value from Jørgensen 1966; Ostapenya et al. 1967; and Platt and Irwin 1973) for the organic samples and the other of 10.18 KJ·g^{-1} P.O.M. (Bayne and Widdows 1978) for the seston samples with a high proportion of detritus. The energy content values calculated by converting the P.O.M. values using a conversion factor of 20.1 KJ·g^{-1} P.O.M. are, on average, 9.5% below those determined by bomb calorimetry and wet oxidation (Table 1).

The results of the analysis of the seston samples on Whatman GF/C filters are presented in Table 3. These values, expressed as per gram dry weight (not ash-free dry weight), are typical of estuarine seston samples with a high proportion of detritus and inorganic material. The conversion of P.O.M. values to Joules, using the conversion factor of 10.1 KJ·g^{-1} P.O.M., considerably underestimates the energy content of the seston samples as determined by wet oxidation.

Discussion

The main reason why the wet oxidation technique is rarely used for determining the energy content of organic samples is that it fails to oxidize fully compounds rich in nitrogen. This contention is supported by experiments using relatively pure nitrogenous compounds. For example, Hughes (1969) found that bovine albumin was only 80% oxidized by potassium iodate and Byers et al. (1978) only obtained 76.6% recovery of *N*-acetyl-D-glucosamine, using potassium dichromate. Although such studies gave accurate information on how the test deals with the more refractory nitrogenous compounds, they, of course, bear little relation to its use in analyzing natural samples where such compounds form only a small proportion of the total organic material. The error associated with the incomplete oxidation of certain nitrogenous compounds will be low in most natural samples. The results of this study found only an average ±3.0% discrepancy between the energy content of the various organics as determined by dichromate wet oxidation and bomb calorimetry. Such accuracy is well within the acceptable range of error for studies of ecological energetics where many parameters e.g. energy lost via catabolism, export from the ecosystem, etc., cannot be easily and directly measured.

A possible reason for poor oxidation of nitrogenous compounds in some studies is the short time allowed for the digestion procedure. Buchanan and Kain (1971) recommended, and Byers et al. (1978) used, heating for 15 min; Hughes (1969) heated for 1 h, and Strickland and Parsons (1972) recommended heating for 1.5 h. However, Maciolek (1962) recommended, on the basis of sequential analysis of carbohydrates, lipids, and crude proteins, that 3 h of heating at 100°C is optimum.

One potential error that is difficult to overcome is the presence of reduced inorganic elements (e.g. ferrous ions), which may be oxidized by the dichromate and hence cause an overestimation of energy content. This was not considered a problem in this study as the seston samples were all from an aerobic environment, but it is a potential source of error in studies involving anoxic sediments. These reduced elements may be oxidized by drying thin layers of the sample at a moderate temperature (50°C) for 24 h in a plentiful supply of air (Newell 1965). Similarly, carbon from coal in the sample will cause the energy content to be overestimated, but this is also a problem in bomb calorimetry and for instrumental C:N analysis (Crisp 1971).

The error associated with determining energy content by wet oxidation may also be less than for biochemical analysis. Crisp (1971) states that the sum of the determined weights of fats, proteins, and carbohydrates is generally less than the original ash-free dry weight. Therefore, he recommends when using biochemical analysis and standard conversion factors to determine energy content, that lipids be determined directly, protein as protein nitrogen × 6.25, and the balance of the ash-free dry weight be considered to be carbohydrate. This, of course, assumes that there is 100% recovery of lipids and proteins. However, as carbohydrate is determined as weight loss on ignition, this hybrid method has no real advantage over the weight loss on ignition technique which is frequently used to estimate the P.O.M. in seston or sediment samples. The weight loss on ignition technique has potential errors, as outlined in the introduction, but if these are controlled (Byers et al. 1978), it can give a useful description of seasonal variation in P.O.M.

In this study, there was poor agreement, for most samples, between the estimates of energy content calculated from P.O.M. values, using conversion factors (derived from the literature) of 20.1 KJ·g^{-1} P.O.M. for predominately organic samples and 10.18 KJ·g^{-1} P.O.M. for seston samples, and the actual energy content, as determined by bomb calorimetry and wet oxidation. This discrepancy is because the conversion factor is dependent on the biochemical composition of the

sample and this varies both with the season and locality. This is perhaps also why there was a difference between the actual energy content values of the plant material and those calculated from the relative proportions of carbon and nitrogen, using the equation derived by Platt and Irwin (1973). However, Platt and Irwin (1973) did stipulate that the relationship between carbon and energy content must be determined separately for each material being tested.

The results of the C:N ratio for both the natural organic and the seston samples yielded typical values (Russell-Hunter 1970). However, there was frequently a discrepancy between the values for the total carbon as determined by instrumental C:N analysis and by the wet oxidation procedure. This is undoubtedly due to the unrealistic factor, derived by Maciolek (1962), used to convert oxidant reduced to carbon content, which is based on the rather tenuous assumption that carbon constitutes 50% of all natural organic material. Therefore, if the carbon content of samples is required, it should either be determined using the C:N analyzer or using wet oxidation and quantifying the CO_2 liberated (Maciolek 1962; Kotsch et al. 1976).

In summary, the quantitative wet oxidation procedure described by Maciolek (1962) and used in this study provides a very fast and accurate method of determining the energy content of organic material and seston samples on glass fiber filters. As the technique does not rely on the combustion of the sample, the proportion of inorganic material present is irrelevant and so this technique is particularly suited to the analysis of seston and nonreduced sediments. The results may be converted directly to energy units and thus yield good information on food availability for the construction of energy budgets. In certain circumstances this technique may overestimate the energy available to animals, e.g. high levels of refractory carbon in the seston. However, this may be overcome by collecting and analyzing, using wet oxidation, the feces produced. This will thus account for, in the energy budget, any non-utilizable food that passes through the animal undigested. An additional advantage of this method is that it yields results in terms of "oxygen demand" of the material and similarly, in many studies of the energy budgets of aquatic animals, metabolic energy requirements are measured as oxygen demand.

Acknowledgments

I am grateful to Dr R. Malouf and Monica Bricelj for helpful discussion at the initiation of this work and to Drs E. Battley and E. Carpenter for the use of equipment. I am also grateful to Dr R. Koehn for the laboratory space in which some of this work was performed and to Mrs C. Newell for the *Tetraselmis suecica* specimens.

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Sublethal Toxicity of Cyanide to Rainbow Trout (*Salmo gairdneri*) at Different Temperatures

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KOVACS, T. G., AND G. LEDUC. 1982. Sublethal toxicity of cyanide to rainbow trout (*Salmo gairdneri*) at different temperatures. Can. J. Fish. Aquat. Sci. 39: 1389–1395.

At sublethal levels cyanide was more potent at lower temperatures. This was verified in 20-d laboratory growth experiments carried out on juvenile rainbow trout (*Salmo gairdneri*) in flow-through aquaria maintained at 6, 12, and 18°C. Cyanide concentrations in the range of 0.005–0.045 mg·L^{-1} HCN significantly reduced growth rates (wet weight, dry weight, and fat), the effective concentrations increasing with temperature. Some adaptation to cyanide seemed to occur as manifested by faster growth rates. Liver glycogen and lactic acid monitored for 20 d in rainbow trout exposed to 0.015 mg·L^{-1} HCN showed an initial shift towards glycolysis, followed by a return to aerobic respiration. This response, however, varied with temperatures. Previous exposure to cyanide markedly reduced swimming performance of the fish tested at their acclimation temperatures, but in the absence of the toxicant. The effect also increased with decreasing temperatures.

Key words: cyanide, sublethal toxicity, rainbow trout, temperature, growth, swimming, liver metabolites

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À des concentrations sublétales, le cyanure est plus toxique à de basses températures. Nous avons vérifié ceci dans des expériences sur la croissance en laboratoire durant 20 jours de jeunes truites arc-en-ciel (*Salmo gairdneri*) dans des aquariums à débit continu maintenus à 6, 12, et 18°C. Les concentrations de cyanure dans la fourchette de 0,005 à 0,045 mg·L^{-1} HCN abaissent significativement les taux de croissance (poids sec, poids humide et graisse), les concentrations effectives augmentant en fonction de la température. Il semble se produire une certaine adaptation au cyanure, qui se reflète dans des taux de croissance accélérés. Les concentrations de glycogène hépatique et d'acide lactique suivies durant 20 jours chez des truites arc-en-ciel exposées à 0,015 mg·L^{-1} HCN accusent un glissement initial vers la glycolyse, suivi d'un retour à une respiration aérobie. Cette réponse varie toutefois en fonction des températures. Une exposition antérieure au cyanure diminue notablement la performance de nage de poissons testés à leurs températures d'acclimatation, mais en l'absence du toxique. L'effet augmente aussi avec des températures décroissantes.

Received December 16, 1980
Accepted June 18, 1982

Reçu le 16 décembre 1980
Accepté le 18 juin 1982

TEMPERATURE acts as a controlling factor on the metabolic rate of fish, influencing the animal's activity in terms of development, growth, and swimming performance (Fry 1971). Because fish in temperate zones must function at different temperatures, the need to consider temperature as a modifying factor of toxicity is important. Whereas some work has been done to evaluate the effect of temperature on the acute toxicity of many compounds, virtually no information exists on the way in which temperature may alter the sublethal effects of toxicants on fish.

The effects of chronic cyanide poisoning on various physiological responses of fish have recently received much attention and were reviewed by Leduc (1977) and Doudoroff (1976, 1980). Cyanide has been shown to reduce seriously the growth of juvenile rainbow trout (*Salmo gairdneri*) exposed to concentrations as low as 0.01 and 0.02 mg·L^{-1} in flow-through aquaria at a temperature of 10.5°C (Dixon and Leduc 1981; Speyer 1975; McCracken and Leduc 1980). Leduc (1966a), however, observed lesser effects of cyanide on growth of coho salmon (*Oncorhynchus kisutch*) and cichlids (black acara, *Cichlasoma bimaculatum*) tested at 16 and 25°C, respectively.

Swimming capacity of fish is temperature-dependent (Warren 1971, p. 106) and has been recognized as a sensitive indicator of chronic poisoning. Neil (1957) showed that

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Printed in Canada (J6330)
Imprimé au Canada (J6330)

cyanide concentrations as low as 0.01 and 0.05 mg·L^{-1} as CN^- reduced the swimming ability of brook trout (*Salvelinus fontinalis*) by as much as 98% after 29 d of exposure at 8°C. Broderius (1970) and Speyer (1975) observed that cyanide concentrations of 0.01 and 0.02 mg·L^{-1} as HCN at 15 and 11°C, respectively, decreased the swimming ability of coho salmon and rainbow trout by about 50%. On the other hand, Leduc (1966a) observed that at 25°C the maximum sustained swimming speed of cichlids was reduced by only 30% after a 24-d exposure to 0.04 mg·L^{-1} HCN.

Although these various studies of the effects of cyanide on growth and swimming performance are difficult to compare because of different experimental procedures, it appears that at higher temperatures the toxicity of cyanide was reduced, as observed by Smith et al. (1978) and Kovacs and Leduc (1982), at lethal levels.

This study was undertaken to determine the effects of temperature on sublethal toxicity of cyanide to rainbow trout as measured by growth and swimming performance at 6, 12, and 18°C. Furthermore, in an attempt to explain the different effects of cyanide at the three temperatures tested, liver glycogen and lactic acid were monitored during sublethal tests.

Material and Methods

Rainbow trout were purchased from La Pisciculture, Mt. Sutton, Sutton, Québec, and were held in running dechlorinated city water at 12°C. The acclimation regime to other temperatures and the experimental conditions and apparatus were identical with those described in Kovacs and Leduc (1982).

Growth Experiments and Swimming Stamina Tests

Two 20-d growth experiments were performed. The first one was designed to determine the food maintenance requirements of rainbow trout at 6, 12, and 18°C, but without cyanide. The second was planned to measure the effects of cyanide on growth at 6, 12, and 18°C, at feeding rates five times the maintenance requirements. In both growth experiments, 25 fish of about 20 g were randomly distributed to four test tanks at each of the three temperatures. Before the first growth experiment, the fish were fed to satiation level once a day during acclimation, whereas before the second, they were fed 1.6, 2.5, and 3.3% of wet body weight per day at 6, 12, and 18°C.

Before the start of the experiments, the fish were not fed for 24 h. They were then lightly anesthetized with 20 mg·L^{-1} MS-222, (tricaine methane sulphonate, Sandoz Corp.), individually branded using the liquid nitrogen method of Mighell (1969), blotted dry, and weighed to the nearest hundredth of a gram.

Twenty fish were returned in each of the test tanks, but five were killed, oven-dried for 7 d at 70°C, and stored to provide preexperimental values of fat and water content. Fat content was measured by ether extraction with a Goldfisch fat extractor using a 2.000-g sample subjected to a 4-h reflux distillation.

In the first experiment, fish kept on four different feeding levels at each of the three temperatures (Table 1) were weighed on day 10, and the feeding levels adjusted. At the termination of the experiments, they were weighed and killed for determination of water and fat content.

In the second experiment, after weighing and branding of the fish, metering of the cyanide solutions from Mariotte bottles (Leduc 1966b) was initiated. Cyanide concentrations were determined daily by the method of Lambert et al. (1975), but never varied by more than 5% from the predicted values. The fish were weighed on day 10 and the feeding levels adjusted. At day 20 the tests were terminated. Five fish from each group were tested for swimming stamina and the others were weighed, killed, and preserved for water and fat content measurements.

Fish of approximately the same length and weight were tested for swimming stamina at the three test temperaures. One fish at a time was inserted in the swimming chamber (Kruzynski 1972), filled with cyanide-free water. The current was turned up to 15 cm·s^{-1} for 3 min, then to 30 cm·s^{-1} for 3 min, finally to 45 cm·s^{-1} for 1 min. Most fish adjusted well and faced the current immediately, but those that failed the training period were ascribed a zero swimming time ability.

After the training period, the current was raised up to 54 cm·s^{-1} and held there for 30 min or fatigue time of the fish, whichever came first.

Liver Metabolites

Glycogen and lactic acid in livers of rainbow trout averaging 28 g were monitored intermittently over a 20-d period, at 6, 12, and 18°C, in the presence of 0.015 mg·L^{-1} HCN or in the absence of cyanide.

Twenty-five trout per tank were acclimated as described previously and fed twice a day as during the second growth experiment. The trout were sampled on four occasions: on day 0 (just before cyanide exposure) and 5, 10, and 20 d after initial cyanide exposure. The cyanide-exposed and control groups were sampled simultaneously.

At each sampling date, five fish were removed from the exposure tanks. The fish were killed, weighed, and measured. Each liver was immediately excised and cut in half, and each half weighed to the nearest 0.001 g, frozen in liquid nitrogen, and stored in a freezer at −20°C. Livers from the five individual fish were pooled at each sampling time. Thus, half the pooled livers were used for glycogen determinations and the other half were used for lactic acid determinations.

The liver samples designated for glycogen determinations were removed from the freezer the next day and were digested in 30% KOH and precipitated with 95% alcohol, according to the method of Good et al. (1933). Glycogen concentrations were determined by the phenol−sulfuric acid colorimetric method of Montgomery (1957).

Liver samples stored for determinations of lactic acid were removed from the freezer within a week after storage. They were crushed to a powder form in a mortar filled with liquid nitrogen. Chilled 8% (w/v) perchloric acid was then immediately added to the liver powder to provide a 4:1 ratio of the final total liquid volume of the sample to its original weight (Bücher et al. 1963). This sample was homogenized for 2 min with a microblade homogenizer (Sorval, Omni-Mixer microhomogenizer) in a 10-mL volume cup. The tissue homogenate

TABLE 1. Comparison of wet weight gains of juvenile rainbow trout (*Salmo gairdneri*) held on different rations at different temperatures for 20 d

Ration (% body wt·d^{-1})	Mean wet weight gain, % (SD)		
	6°C	12°C	18°C
0.0	−8.14 (3.27)	−10.33 (2.35)	−13.21 (1.96)
0.8	12.31 (3.37)		
1.2		14.29 (5.32)	
1.5	28.19 (5.39)		15.51 (4.48)
2.5	29.12 (5.67)	38.05 (6.99)	
3.5			51.13 (7.14)
4.0		60.13 (12.71)	
4.5			65.49 (13.04)
Maintenance ration[a]	0.32	0.48	0.69

[a]Derived from regression equations.

was then cold-centrifugated at 1000 *g* for 15 min. The supernatant was decanted and stored at 2−5°C before a 0.2-mL aliquot was analyzed for lactic acid, determined by the lactic dehydrogenase colorimetric method (Sigma Chemical Co., Kit No. 826−UV, 1968).

Results

Maintenance Requirement

The maintenance level for wet weight of rainbow trout at 18°C was 2.2 times greater than that at 6°C, and it was 1.5 times greater at 12°C than that at 6°C (Table 1). At the rations of 1.5% the trout grew significantly better at 6°C than at 18°C. At a ration of 2.5% the growth of trout was greater at 12°C than at 6°C. At all temperatures, increasing rations seemed to promote fat deposition in the fish and decreased their moisture content. The fat-free dry matter of trout did not seem to be much affected by ration level at any temperature (see Kovacs 1979).

Effect of Cyanide on Growth

All fish survived the test conditions except those that died accidently (Table 2). The fish readily adapted to the test conditions and their behavior appeared normal except at cyanide concentrations of 0.015 mg·L^{-1} HCN at 6°C and 0.030 mg·L^{-1} HCN at 12°C. In these tanks, the fish exhibited rapid, erratic, swimming activity and loss of appetite evidenced by the much longer period of time required for consumption of the entire ration. These behavioral abnormalities occurred only during the first 10 d of exposure.

Table 2 shows growth expressed as the means of specific growth rates (MSGR) of individual fish in percent wet weight gain per day, calculated according to Brown (1957, p. 365). Cyanide generally slowed growth over the 20-d period. In most cases, reduction of growth by cyanide occurred during the first 10 d but was followed by a growth rebound in the second 10-d period. By contrast, at 0.01 mg·L^{-1} HCN and 12°C the fish grew faster throughout the 20-d period. Cyanide had a very marked effect on water and fat contents in fish (Table 3) making it necessary to calculate growth as dry weight gain. These MSGR's transformed into dry weight gains (Table 4) represent true tissue deposition which was more affected by cyanide than were wet weight gains even though only three test groups grew significantly slower than the controls (Students's *t*-test, $P < 0.05$).

Fat gains were calculated by subtracting the pooled fat content of fish at day 0 from the pooled fat content of each group of fish on day 20 and dividing the difference by the number of fish to derive the average absolute fat gained per fish in grams. Absolute fat gains in fish exposed to cyanide at 6 and at 12°C were reduced (Table 4). At 18°C, however, fat gain occurred at the lowest cyanide concentration but decreased at higher concentrations.

The overall effect of cyanide on growing juvenile rainbow trout during the 20-d experimental period is illustrated in Fig. 1 by eye-fitted curves. From these curves, we estimated the threshold levels of cyanide concentrations for each growth parameter and at each of the three test temperatures. Wherever possible, we took the inflection point of the growth curve as being the threshold cyanide concentration. If reductions occurred at the lowest concentration, we reported the thresholds as below those values. The threshold values increased with temperature for all three parameters (Table 5).

Swimming Performance

The distance travelled by each fish was calculated by multiplying swimming time by the velocity (45 cm·s^{-1}) against which the fish was swimming. The results are graphically presented in Fig. 2 by the calculated regression lines of mean log distance travelled versus cyanide concentration at 6, 12, and 18°C.

There were large individual variations in swimming ability, but it was possible to demonstrate some significant relations between temperature and cyanide effects. At any given temperature, the mean log swimming distance of rainbow trout decreased linearly with increasing cyanide concentrations; the slope of the regression lines increased with decreasing temperature, and the three slopes were significantly different from each other ($P < 0.05$, analysis of covariance). This shows that the cyanide effect on the swimming ability of rainbow trout is greater at lower temperatures.

Temperature also influenced the swimming ability of control fish, the effect being most pronounced between 6 and

TABLE 2. Cyanide concentrations, rations, initial mean wet weights, and mean specific growth rates (MSGR) of rainbow trout exposed to various concentrations of cyanide during a 20-d period at different temperatures. (* $P < 0.05$, Student's t-test.)

Temp. (°C)	Ration (% body wt·d^{-1})	Cyanide concn. (mg·L^{-1} as HCN)	Initial mean wet wt (SD) (g)	MSGR: 0–10 d (SD) (% d^{-1})	MSGR: 10–20 d (SD) (% d^{-1})	MSGR: 0–20 d (SD) (% d^{-1})
6	1.5	0.000	17.43 (0.94)	1.10 (0.34)	1.23 (0.30)	1.16 (0.18)
		0.005	15.70 (0.88)	1.07 (0.39)	1.33 (0.38)	1.19 (0.26)
		0.010	16.61 (0.92)	1.02 (0.62)	1.26 (0.64)	1.14 (0.46)
		0.015	16.97 (0.99)[a]	0.50*(0.63)	1.31 (0.55)	0.91*(0.53)
12	2.5	0.000	19.34 (0.94)	1.59 (0.46)	1.76 (0.53)	1.67 (0.39)
		0.010	17.16 (0.91)	1.71 (0.45)	1.83 (0.29)	1.77 (0.26)
		0.020	18.29 (0.87)	1.13*(0.71)	1.93 (0.58)	1.53 (0.53)
		0.030	17.81 (0.84)	0.39*(0.89)	1.26*(0.95)	0.82*(0.82)
18	3.3	0.000	19.02 (0.85)	1.99 (0.37)	2.00 (0.29)	1.99 (0.27)
		0.015	19.79 (0.97)	1.99 (0.36)	1.96 (0.38)	1.98 (0.28)
		0.030	19.02 (0.93)	1.85 (0.32)	1.98 (0.51)	1.91 (0.33)
		0.045	19.21 (0.86)[b]	1.00*(0.87)	1.34*(0.67)	1.17*(0.72)

[a]One fish jumped out of the tank overnight.
[b]Four fish died of cyanide poisoning when the dilution water was accidentally shut off for about 60 min.

TABLE 3. Body composition (average of pooled samples) of juvenile rainbow trout (based on wet weight), at the beginning and at the end of a 20-d period, exposed to various levels of cyanide at different temperatures.

Temp. (°C)	HCN (mg·L^{-1})	Water (%)	Fat (%)	Fat-free dry matter (%)
6	Preexperimental	80.4	1.6	18.0
	0.000	79.1	2.6	18.4
	0.005	79.9	2.1	18.0
	0.010	79.9	2.1	18.0
	0.015	81.4	1.7	17.1
12	Preexperimental	79.5	1.7	18.8
	0.000	77.5	4.0	18.5
	0.010	77.5	3.7	18.9
	0.020	77.7	3.3	19.0
	0.030	80.2	2.2	19.1
18	Preexperimental	77.7	2.8	19.5
	0.000	74.9	4.6	20.5
	0.015	74.4	4.8	20.8
	0.030	73.3	4.4	20.8
	0.045	75.7	3.7	20.6

12°C, but there was a statistically significant difference ($P < 0.02$) only between results obtained at 6 and 18°C.

LIVER METABOLITES

Changes in glycogen and lactic acid levels in control and cyanide exposed trout over a 20-d period at 6, 12, and 18°C are shown in Table 6.

In the cyanide-treated group, glycogen levels dropped after 5 d exposure at 6°C, but were near normal levels by day 20. At 12°C, glycogen concentration decreased around day 10 but again returned to a near-normal level by day 20. Comparing average values of glycogen between control and treated groups, it appears that glycogen levels were decreased by cyanide at 6 and 12°C, but not at 18°C. The decrease was greatest at 6°C.

TABLE 4. Mean specific grow rate (MSGR) based on dry weights and absolute fat gain by rainbow trout during a 20-d exposure to various cyanide concentrations at different temperatures. (* $P < 0.05$.)

Temp. (°C)	HCN (mg·L^{-1})	MSGR: 0–20 d (% d^{-1}) (SD)	Absolute fat gain 0–20 d (g)
6	0.000	1.49 (0.18)	0.295
	0.005	1.35 (0.30)	0.177
	0.010	1.22*(0.48)	0.168
	0.015	0.71 (1.67)	0.071
12	0.000	2.12 (0.43)	0.714
	0.010	2.19 (0.35)	0.571
	0.020	1.93 (0.50)	0.489
	0.030	0.63*(0.81)	0.147
18	0.000	2.61 (0.27)	0.770
	0.015	2.61 (0.36)	0.854
	0.030	2.53 (0.33)	0.676
	0.045	1.60*(0.72)	0.372

At 6°C, cyanide produced changes in lactic acid that were inversely related to those of glycogen; lactic acid rose sharply on day 5, but returned to normal by day 10. At 12 and 18°C, liver lactic acid concentrations in control as in cyanide-exposed fish were similar.

Discussion

Cyanide (HCN) at sublethal levels is more toxic to trout at lower temperatures as exemplified by our observations of growth and swimming performance. The responses to cyanide at different temperatures must reflect temperature-related changes in the physiological and biochemical state of the fish that occur during temperature acclimation (Hochachka and Somero 1971).

The greater toxicity of cyanide at lower temperatures could be the result of several processes acting together on the fish.

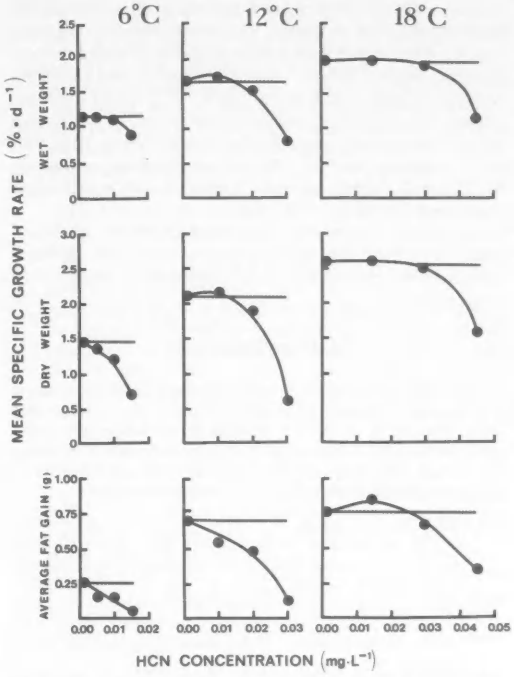

FIG. 1. The relationship between wet weight, dry weight, and fat gain of juvenile rainbow trout and concentration of cyanide to which they were exposed during 20-d experiments under flow-through conditions at three temperatures.

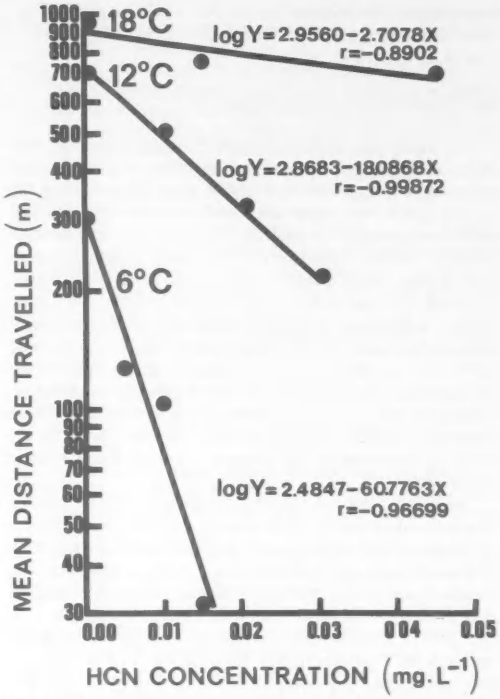

FIG. 2. Regressions between mean distance travelled by juvenile rainbow trout and the concentrations of cyanide to which they were exposed for 20 d at three temperatures.

TABLE 5. Threshold concentrations of cyanide ($mg \cdot L^{-1}$ HCN) that produced no effect on the growth of rainbow trout during a 20-d exposure in flow-through aquaria at different temperatures.

Temp. (°C)	Wet weight	Dry weight	Fat
6	0.01	<0.005	<0.005
12	0.015	0.010	<0.01
18	0.030	0.030	0.025

TABLE 6. Liver glycogen and lactic acid levels of juvenile rainbow trout during exposure to 0.015 $mg \cdot L^{-1}$ HCN at different temperatures.

Day of exposure		Control			Treated		
		6°C	12°C	18°C	6°C	12°C	18°C
		Liver glycogen levels ($mg \cdot g^{-1}$)					
0		57	69	56	52	63	55
5		47	72	54	12	66	62
10		64	79	54	16	48	61
20		70	83	56	47	67	47
	Average	60	76	55	32	61	56
		Liver lactic acid levels ($mg \cdot 100\ g^{-1}$)					
0		22	26	27	19	28	27
5		15	28	30	34	27	25
10		16	21	26	17	22	30
20		18	18	21	19	21	25
	Average	18	23	26	22	24	27

Qualitative changes of cytochrome oxidase were suggested by Ekberg (1962) and Webb (1963) to explain the greater sensitivity of poikilotherms to cyanide at low temperatures. Metabolic differences due to thermal history may also explain temperature-related changes in the toxicity of cyanide. Low temperatures depress the Krebs cycle (Dean 1969) and ATP synthesis which is further reduced by cyanide resulting from the inhibition of cytochrome oxidase. In our study, the increased glycolitic activity observed at 6°C (Table 6) suggests cytochrome oxidase inhibition at 0.015 $mg \cdot L^{-1}$ HCN, a concentration which reduced growth at 6°C. This level, however, had no effect on growth at 12 and 18°C.

Cyanide may be detoxified by rhodanese which has been identified in many animals including fish (Westley 1981; Sido and Koj 1972; Schievelbein et al. 1969). The enzyme transforms HCN into SCN^- which is known to reduce thyroxine synthesis in mammals (Bourdoux et al. 1978). Thyroxine promotes growth in trout, (Narayasingh and Eales 1975), and its need is greater at lower (7°C) than higher (15−19°C)

temperatures (Leatherland et al. 1977). Consequently, cyanide would have a greater effect on the growth of trout at 6°C than at 12 and 18°C.

Growth Rebound and Stimulation

The depressing effects of cyanide on growth were very prominent during the first 10 d of exposure at all three temperatures, but cyanide-exposed fish grew faster during the second half of the experiments and in some cases attained greater weights than the controls (Table 4). The rebound from an initial growth depression due to cyanide poisoning seems to be related to the extent of the original depression, a phenomenon also noted by Leduc (1966a), Dixon and Leduc (1981), and Speyer (1975). Measurements of liver glycogen and lactic acid have shown that at 6°C, cyanide (0.015 $mg \cdot L^{-1}$ HCN) initially stimulated glycolysis, but the fish apparently returned to their normal aerobic metabolism by day 10, at which time growth rates accelerated. These responses suggest an overcoming of the toxic effect of cyanide by returning to the greater energy-releasing aerobic metabolism.

Cold-acclimated fish tend to overcome the decrease in cytochrome oxidase activity by producing more enzyme (Caldwell 1969; Freed 1965; Shaklee et al. 1977). If such a response can be induced by low temperature, then cyanide, by depressing metabolic rate, may evoke a similar response; this explains why growth rebound was more prominent at 6°C than at 12°C and greater at 12°C than at 18°C. The level of oxidative enzymes in cyanide-exposed fish has not been measured; however, guinea pigs adapted to low oxygen (4200-m altitude) have higher succinoxidase and cytochrome oxidase levels than controls kept at sea level (Tappan et al. 1957). Detoxification of cyanide by rhodanese, which could be a delayed response, would provide the fish with higher cytochrome oxidase levels and lead to a more pronounced growth rebound.

Some cyanide-stimulated growth responses were observed at 12 and 18°C (Fig. 1) throughout the experimental period. Similarly, cyanide at 0.01 $mg \cdot L^{-1}$ HCN promoted higher growth rates and production of young chinook salmon (*Oncorhynchus tsyawytscha*) held in an artificial stream in a current of 24 $cm \cdot s^{-1}$ (Negilsky 1973). The growth of small, 5-g rainbow trout swimming at 12 $cm \cdot s^{-1}$ and exposed to 0.01 $mg \cdot L^{-1}$ HCN at 10°C was also stimulated (McCracken and Leduc 1980). Low concentrations of cyanide (0.015 $mg \cdot L^{-1}$ HCN) can enhance growth in fish with higher metabolic rate due to either higher temperature, current, and/or size.

Ecological Significance

After a 20-d exposure to sublethal cyanide, the swimming ability of rainbow trout was reduced at all cyanide concentrations, the effect increasing at lower temperatures. Although cyanide-exposed fish had returned to normal or near normal growth rates, their swimming impairment suggests biochemical disturbance and perhaps tissue damage as observed by Dixon and Leduc (1981).

At low water temperatures (4–5°C), under conditions where metabolism is depressed, fish are under some stress to maintain their life processes. This is evidenced by a greater water content of fish, less food availability in nature, greater specific dynamic action, lower assimilation, and food conversion efficiency (Warren and Davis 1967). Under such conditions, another stressor such as cyanide would have a serious effect on fish production and even on long-term survival. Our study indicates that at 6°C, a concentration as low as 0.005 $mg \cdot L^{-1}$ HCN can cause marked reduction in fat synthesis and swimming performance.

In Canadian waters, low water temperatures prevail. Therefore, for a more realistic appraisal of our water pollution problems, toxicity to fish at low temperatures needs to be evaluated.

Acknowledgments

This project was supported by a Department of Indian and Northern Affairs research contract (0SU5-0010). The department of Education of the Province of Quebec also provided financial support through a graduate scholarship. We extend special thanks to Dr S. d'Apollonia for her help with the biochemical tests and to Mr T. Jacobovic for his aid in weighing the fish and in the swimming stamina tests.

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Measurements of Growth and Consumption of Sauger (*Stizostedion canadense*): Implication for Fish Energetics Studies[1]

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MINTON, J. W., AND R. B. MCLEAN. 1982. Measurements of growth and consumption of sauger (*Stizostedion canadense*): implication for fish energetics studies. Can. J. Fish. Aquat. Sci. 39: 1396 – 1403.

The energy budget of sauger (*Stizostedion canadense*), in Watts Bar Reservoir, Tennessee, was studied over a 17-mo period. Field determinations of consumption and laboratory determinations of metabolism were incorporated into the balanced energy equation of Winberg to predict growth in energy value of sauger in age-classes I^+ and II^+. The equation included energetic costs of specific dynamic action and the metabolic efficiency penalty of metabolizing body energy stores. Observed and predicted growth during the warm months, March through October, agreed within 2.5 – 9.6% for the I^+ and II^+ age-classes, respectively. Observed and predicted growth over the winter for the only age-class modeled, the I^+, did not agree. High food consumption during winter did not result in the amount of growth expected, suggesting that the physiological assumptions of the balanced energy equation are invalid for describing the winter energetics of sauger. We emphasize the potential error in inferring consumption from growth and metabolism.

Key words: sauger, *Stizostedion*, bioenergetics, feeding, growth, modeling

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Le budget énergétique du doré noir (*Stizostedion canadense*) dans le réservoir de Watts Bar, dans le Tennessee, a été étudié sur une période de 17 mois. Les mesures de consommation sur le terrain et celles du métabolisme en laboratoire ont été incorporées dans l'équation énergétique équilibrée de Winberg afin de prédire la croissance en termes d'énergie chez des dorés de classes d'âge I^+ et II^+. L'équation inclut les coûts énergétiques d'une action dynamique spécifique et la diminution du rendement énergétique résultant de l'utilisation des réserves corporelles. La croissance observée et prédite pendant les mois chauds (mars à octobre inclusivement) s'accorde avec écarts de 2,5 à 9,6 % pour les classes d'âge I^+ and II^+ respectivement. Par contre, la croissance observée en hiver chez la seule classe d'âge introduite dans le modèle, la classe I^+, ne s'accorde pas avec celle prédite. Malgré une forte consommation d'aliments en hiver, la croissance anticipée ne se matérialise pas, ce qui donne à penser que les hypothèses physiologiques d'une équation énergétique équilibrée sont invalides quand il s'agit de décrire l'énergétique hivernale du doré noir. Nous soulignons le danger d'erreur à déduire la consommation à partir de la croissance et du métabolisme.

Received June 19, 1981
Accepted June 7, 1982

Reçu le 19 juin 1981
Accepté le 7 juin 1982

THE value of bioenergetics models in describing energy budgets and production dynamics of fish has been widely recognized since Winberg (1956) published the "balanced energy equation." There has been particular emphasis on estimating food consumption for populations and making inferences concerning community trophic dynamics. Only a few of these investigators (Backiel 1971; Healey 1972; Adams 1976) have measured consumption independently to attempt to verify their model predictions; they have had mixed success. These energy budgets omitted adjustments for certain phenomena currently considered to affect fish energetics. These are the energetic cost of assimilating and processing foods (specific dynamic action), which is about 15% of intake energy (Webb 1978; Brett and Groves 1979; Jobling 1981), and the efficiency penalty of metabolizing stored energy (Kleiber 1961; Niimi and Beamish 1974). In this study we use independent estimates of consumption and metabolism and the most current bioenergetic modeling assumptions to predict growth of

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Printed in Canada (J6548)
Imprimé au Canada (J6548)

sauger (*Stizostedion canadense*) in Watts Bar Reservoir, Tennessee.

Materials and Methods

Watts Bar Reservoir is a 13.3-km^2 impoundment created in 1942 by the construction of a Tennessee Valley Authority dam at Tennessee River Mile 529.9. The reservoir is characterized by broad reaches and shallow flats about the main channel and by narrow riverine topography in two of the three tributaries. It is a mesotrophic system with a diversity of native and introduced fish species.

Sauger were sampled from the reservoir for analysis over a 17-mo period. Five 38-m experimental gill nets placed in five locations in the upper riverinelike and lower lakelike portions of the reservoir were fished for 24 h biweekly during November 1978 to March 1980. Surface and bottom-water temperature, dissolved oxygen, and transparency measured with a Secchi disk were recorded at each sampling for all stations. All fish captured were analyzed in the laboratory within 3 h or were frozen for analysis at a later date.

On seven selected dates over the 17-mo study, sauger were captured in a special sampling procedure for consumption rate analysis. Nets were fished for 2-h periods at intervals shorter than required, for sauger digestion to reach 90% completion. Thus for the population, a 24-h feeding regime was represented, and average time of capture was known to within 1 h. Fish were immediately frozen on dry ice for transport to the laboratory.

Total length, wet weight, sex, gonad weight, and stomach contents were determined in the laboratory. Visceral fat tissue, which occurred in a discrete mass attached to the pyloric ceca, and gonad tissue were carefully removed by hand for weighing. Scale samples were removed from all sauger and a representative subsample of the total catch was selected for calorimetric analysis.

Length, weight, and identity of diet items were used in determining consumption rates. Partially digested fish found in the stomachs were identified to genus or species, if possible, by examination of taxa-specific structures resistant to digestion such as gizzards, otoliths, and spines. Total length, standard length, or backbone length was measured, depending on the degree of digestion of the prey item. Given the identity of prey eaten and one of the length measurements, undigested weight of the prey was estimated by length to weight regressions determined from prey species captured in gill nets. Occasionally fish had regurgitated food remnants in the mouth or distended but empty stomachs evidencing regurgitation. Such fish were omitted from diet analysis.

The average individual daily ration of the sauger population on the special sampling dates was then estimated by the equation of Popova and Sytina (1977):

$$R = \sum_{i=1}^{V} \frac{S_i/n_i}{V} \cdot \frac{n}{N},$$

where R = average daily ration of the population (g prey/g predator), V = number of days required for digestion to reach 90% completion, S_i = original undigested weight of prey items eaten by n_i predators, on day i (g prey/g predator), n_i = number of fish in the sample which contained food eaten on day i, n = number of fish in the sample which contained food, and N = number of fish in the sample. When time required for digestion to reach 90% completion equalled 24 h, the equation can be simplified to: $R = S_i/N$.

Data of Swenson and Smith (1973) and McGee (1978) were used to generate a regression of time for digestion to reach 90% completion versus water temperature:

$$\ln y = 4.6807 - 0.1046T,$$

where y = time (h) required for digestion to reach 90% completion, T = water temperature (0°C). The coefficient of determination for this regression equation was 0.92. Swenson and Smith (1973) determined sauger digestion rates at 14.5 and 20°C, and McGee (1978) made similar determinations at 5, 10, and 15°C, representing nearly the entire annual temperature range at our sampling locations (4.7−22.0°C). We interpolated the 90% completion point of digestion from the published figures of Swenson and Smith (1973) and McGee (1978), who determined at a given temperature, digestion progresses linearly with time until nearly complete. The 90% digestion period was utilized because the length of the final digestion phase, the residual phase, is quite variable (Windell 1966). Digestion time was calculated from the studies for meals of 35 mg/g, close to the mean size of Watts Bar sauger (38.8 mg/g).

To avoid any possible bias associated with nonregular feeding patterns (Darnell and Meirrotto 1962), blocks of 24 h were considered in determining consumption rates. For instance, if water temperature resulted in 90% digestion period of between 24 and 48 h, only stomach contents less than or equal to 24 h old were included in the analysis. If the 90% digestion period was between 12 and 24 h, two gillnet samples were collected 12 h apart, and only stomach contents which were less than or equal to 12 h old were included in the analysis of each half of the day's feeding estimate. Residence time of stomach contents was proportional to degree of digestion because of the linear digestion progress. Thus, time of ingestion could also be calculated to determine the major feeding periods of sauger.

The feeding periodicity of the population was determined. For samples in which the 90% complete digestion time was at least 24 h, numbers of feeding incidents were summed over four 6-h periods which roughly corresponded to dawn, day, dusk, and night cycles. These were 02:00 to 08:00, 08:00 to 14:00, 14:00 to 20:00, and 20:00 to 02:00. The distribution of sums was compared with the Chi-square.

Further consumption estimates were derived from gut content data of sauger caught in 24-h net sets. These data could not be used to calculate daily ration directly, because the exact time of capture within the 24-h net set was unknown, and digestion of stomach contents continued after the fish were caught. However, these data did serve as an index of feeding which could be correlated with daily ration. The percent of these fish which contained food in their stomachs (feeding index) was adjusted for effects of temperature on stomach-emptying rates by the formula:

$$\text{AFI} = \text{FI} \cdot 24/\text{HT},$$

where AFI = adjusted feeding index, FI = feeding index,

and HT = number of hours to 90% completion of digestion of a 35-mg prey/g predator meal at the ambient temperature. The daily ration determinations (R) were then regressed on the adjusted feeding indices calculated from fish caught on the same or consecutive dates. The resulting regression equation was used to estimate daily ration from each adjusted feeding index.

Daily ration in terms of weight ingested was converted to joules ingested. The energy equivalent per gram of tissue of the forage species was determined by bomb calorimetric analysis of representative individuals (Phillipson 1966). The sum of the products of the weight of each forage species ingested and its mean energy equivalent yielded the sauger's energy ration.

The relationship of the sauger's standard metabolic rate to temperature was determined by respirometry. The standard oxygen consumption rate of each of two groups of four sauger (mean weights 393 and 397 g) was determined in a darkened 780-L sealed holding chamber by measuring the dissolved oxygen decline rate (Minton 1981). All food was removed for a period sufficient for complete digestion prior to experimentation. During experimentation, the oxygen tension was observed to decline linearly, suggesting that only standard metabolism was measured (Fry 1957). Time-lapse photography of control fish showed them to stay motionless on the botton during low light conditions.

Oxygen consumption of the first group was tested from 17.4 to 5°C at ~ 2°C intervals. The second group was tested from 15°C to 25°C at the same intervals. Tank temperature was adjusted at 1°C per day to avoid thermally stressing the fish.

The relation between metabolism, temperature, and body weight was formulated by combining the empirically determined metabolism−temperature relationship with the general teleost metabolism−weight relationship. The standard metabolic rate of fish is proportional to approximately the 0.85 power of weight at all temperatures (Winberg 1956; Paloheimo and Dickie 1966; Ricker 1973; Brett and Groves 1979). Kelso (1972) calculated that the exponent for walleye (*Stizostedion vitreum vitreum*) was 0.84.

Beginning in October 1979, scale samples were removed from all sauger dissected and were analyzed by the method of Tesch (1971) to determine the fishes' ages and the radius to each scale annulus. Geometric means regression analysis (Ricker 1973) was used to estimate the total length of the fish at each annulus formation. Because scales were not analyzed prior to October it was necessary to assume that annulus formation in 1979 occurred at the same period as in 1980 (mid-March). Water temperature regimes were similar in the two seasons. Sauger captured in March 1979 or any prior month in the winter no-growth period, whose total length fell within ±1 SD of the mean predicted length of a year-class, were assumed to be of that year-class. The mean and standard deviations of lengths of the year-classes were also determined in October 1979, when scale samples were first analyzed. Ranges of standard deviations between March and October were then linearly interpolated for the intervening months to arbitrarily designate the size ranges of the year-classes in each month. We believe this procedure to be conservative and introduced little bias. No length ranges (±1 SD) of different ages overlapped, nor did any individual of known age fall into the length range (±1 SD) of another.

Energetic analysis was performed on at least two representatives of adult sauger of each sex randomly selected monthly or bimonthly. Visceral fat and gonads were analyzed independently of body carcass if they were more than 1% of body weight. The total energy value of individuals from the monthly sample was estimated as the sum of the products of the body component weights and the mean energy values per gram of the subsamples' components.

Total growth over a period was determined as the difference between the observed final and initial mean energy values of individuals of a year-class. For spring and summer growth the gonadal tissue energy value was omitted in calculating the spring value, as this store is lost to the fish upon spawning in early spring.

Energetics Model

The model is a sequential operation of the basic energy equation of Winberg (1956):

$$Q_{G_i} = (1 - P) \cdot Q_{R_i} - Q_{S_i},$$

where Q_{G_i} = somatic and gonadal energy growth over a time interval, i; P = fraction of Q_{R_i} lost due to apparent specific dynamic action (SDA) and egestion and excretory products; Q_{R_i} = total energy ration over a time interval, i; and Q_{S_i} = total energy requirement of standard metabolism over a time interval, i.

Calculations were performed separately on the two abundant year-classes. The weight of an average individual was estimated from its predicted energy value by the observed mean monthly weight−energy equivalent for the year-class. The mean monthly ration per gram was multiplied by the individual's predicted weight to determine its total monthly ration. The fraction P was assumed to be 0.35, the sum of fecal and nonfecal energy losses observed in most piscivorous fish (0.20) (Brett and Groves 1979) and apparent (SDA) energy losses (0.15) (Webb 1978; Brett and Groves 1979; Jobling 1981).

The energy requirement of standard metabolism (Q_S) was estimated by a submodel which related metabolism to body weight and the mean monthly water temperature. Locomotor metabolic cost was assumed to be minimal in sauger (Kitchell et al. 1977) and was not included in the predicting model.

The total ration was reduced by the proportionality constant and then by the cost of standard metabolism to predict the energy gain over a month. If the model predicted a net energy loss for a period, an additional energy loss of 7.5% of the negative balance was assessed to reflect the physiological cost of utilizing stored energy to meet metabolic requirements (Niimi and Beamish 1974).

Results

A total of 1114 sauger were caught over the 17-mo sampling period. Catches were sufficient (designated as 10 fish per month minimum) for energetic and dietary analysis of two year-classes, 1978 and 1977, which were aged I^+ and II^+, through the latter 12 mo of the study.

TABLE 1. Regression equations relating the weight of a fish to its length for the major prey species and energy values for those prey.

Species	Regression equation[a,b]	r^2	N[c]	Energy value[d]	N[e]
Bluegill	$\log W = 3.0366 \cdot \log BL - 1.2144$	0.89	22	$4.44 \cdot 10^6$	29
(*Lepomis macrochirus*)	$\log W = 3.5241 \cdot \log SL - 2.0948$	0.96			
	$\log W = 3.6144 \cdot \log TL - 2.5271$	0.95			
Drum	$\log W = 3.3241 \cdot \log BL - 1.6266$	0.87	34	$5.23 \cdot 10^6$	7
(*Aplodinotus grunniens*)	$\log W = 3.2962 \cdot \log SL - 1.9127$	0.94			
	$\log W = 2.6661 \cdot \log TL - 1.6055$	0.94			
Logperch	$\log W = 1.7380 \cdot \log BL - 0.3482$	0.70	26	$5.44 \cdot 10^6$	7
(*Percina caprodes*)	$\log W = 2.5708 \cdot \log SL - 1.3928$	0.82			
	$\log W = 2.6146 \cdot \log TL - 1.6197$	0.83			
Gizzard shad (*Dorosoma cepedianum*	$\log W = 2.6630 \cdot \log BL - 1.2132$	0.92	71	$4.89 \cdot 10^6$	66
and threadfin shad *D. petenense*)	$\log W = 2.9665 \cdot \log SL - 1.6982$	0.96			
	$\log W = 3.0027 \cdot \log TL - 2.0652$	0.96			

[a] W = total wet weight in grams, BL = backbone length in centimetres, SL = standard length in centimetres, and TL = total length in centimetres.

[b] Log transformation of data yielded the best regression equation as determined by distribution of residual sums of squares.

[c] Sample size for regression analysis.

[d] Joules per gram wet weight.

[e] Sample size for energetic analysis.

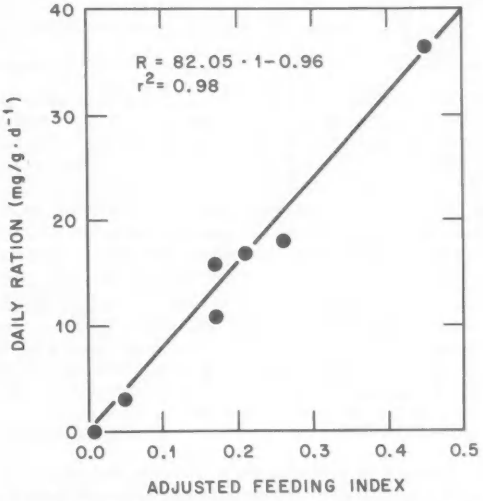

FIG. 1. Relationship between paired observations of adjusted feeding index of sauger caught in 24-h net sets and daily ration of sauger caught on seven separate dates.

Daily ration ranged from 0 mg/g (0 J/g) in March 1980 to 36.7 mg/g (179.6 J/g) in December 1979. Table 1 gives the length, weight, and energy value relationships of prey which were used in the ration determination.

The regression of daily ration on the adjusted feeding indices of the 24-h samples indicates a highly significant relationship ($P < 0.001$) (Fig. 1). Based on this relationship daily ration was estimated from the adjusted feeding indices

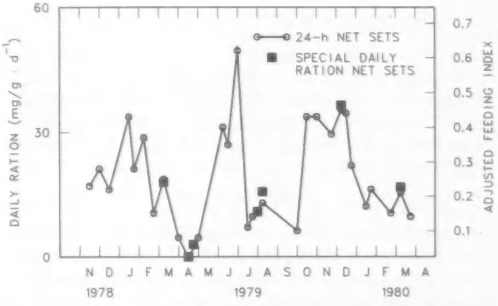

FIG. 2. Comparison of daily ration and adjusted feeding index. Squares represent the daily ration of sauger caught in the seven special netting efforts. Circles represent adjusted feeding index of sauger caught in the 24-h net sets.

for the entire study period. The seven daily ration determinations spanned most of the time period of the study and nearly the entire range of feeding rates observed (Fig. 2).

The daily ration data subdivided into four 6-h intervals showed the population's feeding to be uniform over the diel periods (Chi-square test; $P > 0.75$). Thus the adjusted feeding index of the 24-h sample should be unbiased with respect to feeding periodicity.

The relationship between temperature and the standard oxygen consumption rate of sauger (Fig. 3) was well described ($r^2 = 0.91$) by the regression equation:

$$Q_s(\text{mg } O_2 \cdot h^{-1} \cdot g^{-1}) = 0.0233 \cdot 10^{0.03T},$$

where T = temperature in (°C).

Given the metabolism–weight relationship, the form of the metabolism equation becomes:

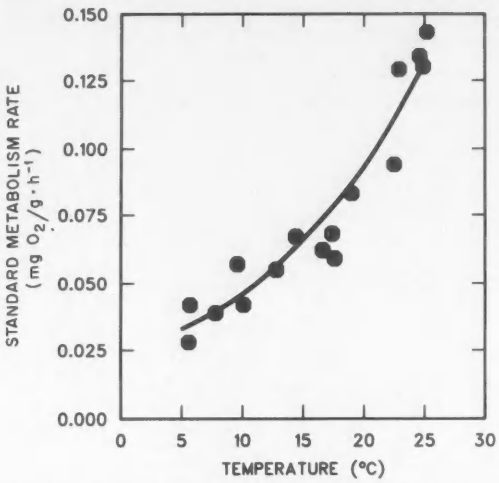

FIG. 3. Observed standard metabolic rate of sauger at various temperatures in milligrams of oxygen consumed per gram of fish per hour. The curve represents the log-transformed regression curve fitted to the data by the method of least squares.

$$Q_s(\text{mg } O_2/\text{h}) = X \cdot 10^{0.03T} \cdot W^{0.85}$$

where W = weight (g), and X = regression coefficient.

Because the metabolic rate of a 395-g sauger (mean weight of experimental animals) was predicted by our weight-specific metabolism regression, we solved for the coefficient of the regression required to balance the complete metabolism equation. The complete regression equation is:

$$Q_s(\text{mg } O_2/\text{h}) = 0.057 \cdot 10^{0.03T} \cdot W^{0.85}$$

Respiration metabolism was converted to energetic metabolism using the conversion factor 14.07 J per gram oxygen respired (Phillipson 1966). The standard energetic metabolism equation was then expressed as:

$$Q_s(\text{J/h}) = 0.803 \cdot 10^{0.03T} \cdot W^{0.85}$$

No year-class grew significantly in length over the winter from October 1979 to February 1980 (one-way ANOVA; $P > 0.05$). However, the energy value per unit weight of both year-classes increased significantly over the season (ANOVA, $P < 0.05$) (Fig.4). These changes resulted from significant increases in fat and gonad weight to body weight ratios and energy value per gram of carcass over the winter period (Minton 1981). In 1978–79 the total energy value per gram peaked in early spring for both year-classes, and in 1979–80 it was at its highest value when sampling was discontinued in late winter. The changes in energy value tracked changes in fat and gonad weight to body weight ratios because the mean energy value per gram wet weight of fat and gonad ($32.2 \cdot 10^3$ and $17.6 \cdot 10^3$ J, respectively) was much higher than that of the carcass ($5.9 \cdot 10^3$ J). Weight-specific energy changes were factored into the calculated growth over the seasons (Table 2).

The gross efficiency for the observed growth in energy

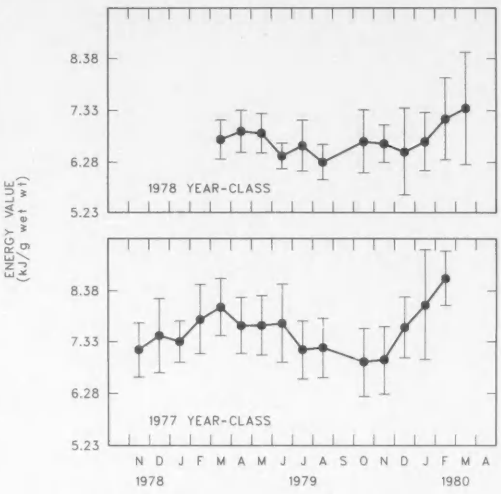

FIG. 4. Calculated monthly means and standard deviations of the energy values of individuals of the 1978 year-class (*upper* graph) and 1977 year-class (*lower* graph).

TABLE 2. Average measured energy values of individuals of the 1978 and 1977 year-classes of sauger at critical times in the study period.

Date	Energy value (J)	
	1978 year-class (n)	1977 year-class (n)
November 15, 1978	$0.938 \cdot 10^6$ (12)	$2.698 \cdot 10^6$ (20)
March 15, 1979	$0.947 \cdot 10^6$ (11)	$3.640 \cdot 10^{6a}$ (31)
October 15, 1979	$2.446 \cdot 10^6$ (22)	$5.102 \cdot 10^6$ (15)
March 15, 1980	$3.103 \cdot 10^6$ (23)	(7)[b]

[a]Energy value not including gonads is $3.460 \cdot 10^6$.
[b]Insufficient sample size for accurate estimate.

value of the sauger based on the observed weight-specific ration and linearly interpolated growth was calculated to be 29.4% for the 1978 year-class in the growing season, 8.0% in the winter of 1979–80, and 20.4% for the 1977 year-class in the growing season.

ENERGETICS MODEL

Sample sizes were adequate to model the energetics of the 1978 year-class over both the growing season and winter and for the 1977 year-class for the growing season only. As an example of the model's application, the results of the calculations for the 1978 year-class over the growing season are presented in Table 3. The algorithm for the model is:

$$G = 0.65\,(A \cdot D \cdot E) - (A \cdot 0.0046 \cdot 10^{0.03}\,(F) \cdot D^{0.85})$$

where the variables A through G refer to the columns of parameters in Table 3, and:

$$D = B/C,$$
$$B_{i+1} = B_{i+G},$$

Predicted energy gain over the warm weather growth period

TABLE 3. Calculated results of the bioenergetics model for the period March 15, 1979 to October 15, 1979, for the 1978 year-class.

Initial date of interval	Days in interval	Predicted body energy value (J)	Observed body energy equivalent (J/g)	Predicted body weight (g)	Observed energy ration ($J \cdot g^{-1} \cdot d^{-1}$)	Observed mean monthly temp. (°C)	Predicted energy growth in interval (J)
March 15, 1979	16	$9.47 \cdot 10^5$	$6.95 \cdot 10^3$	136.3	29.18	5.7	$1.34 \cdot 10^4$
April 1	30	$9.60 \cdot 10^5$	$6.67 \cdot 10^3$	143.4	3.64	11.3	$-8.16 \cdot 10^4$
May 1	31	$8.79 \cdot 10^5$	$6.44 \cdot 10^3$	136.3	123.43	16.6	$21.98 \cdot 10^4$
June 1	30	$10.99 \cdot 10^5$	$6.10 \cdot 10^3$	180.1	247.02	19.6	$68.20 \cdot 10^4$
July 1	31	$17.81 \cdot 10^5$	$6.25 \cdot 10^3$	285.4	106.30	19.6	$33.74 \cdot 10^4$
Aug. 1	31	$21.18 \cdot 10^5$	$6.23 \cdot 10^3$	339.5	46.31	21.3	$-4.40 \cdot 10^4$
Sept. 1	30	$20.74 \cdot 10^5$	$6.40 \cdot 10^3$	323.8	76.74	23.0	$9.80 \cdot 10^4$
Oct. 1	15	$21.72 \cdot 10^5$	$6.59 \cdot 10^3$	329.6	108.31	19.2	$19.72 \cdot 10^4$
Oct. 15	0	$23.70 \cdot 10^5$	$6.59 \cdot 10^3$	359.6			

TABLE 4. Comparison of observed and predicted growth of two year-classes of sauger in Watts Bar Reservoir over selected intervals of the study period.

Time period	Year-class	Observed growth (J)	Predicted growth (J)	Observed gross efficiency (%)	Predicted gross efficiency (%)
March 15–Oct. 15, 1979	1978	$1.498 \cdot 10^6$	$1.422 \cdot 10^6$	29.4	30.6
	1977	$1.642 \cdot 10^6$	$1.860 \cdot 10^6$	20.4	24.1
Oct. 15, 1979–March 15, 1980	1978	$0.658 \cdot 10^6$	$5.725 \cdot 10^6$	8.0	52.9
	1977[a]				

[a]Sample not large enough for analysis.

(March 15–October 15, 1979) (Table 4) was close to the observed gain for both age-classes of sauger. The model predicted, in mid-October 1979, that the 1978 year-class would have a mean energy value of $2.400 \cdot 10^6$ J, representing a $1.372 \cdot 10^6$ J energy gain since March 15, 1979. This predicted growth was 2.5% lower than the observed growth of $1.407 \cdot 10^6$ J. The calculation for the 1977 sauger year-class predicted a final value of $5.26 \cdot 10^6$ J, a $1.800 \cdot 10^6$ J energy gain over the period, 9.6% higher than the observed $1.642 \cdot 10^6$ J gain. The predicted energy surplus over the 1979–80 winter period for the 1978 sauger year-class was quite high (Table 4); the model predicted a $5.725 \cdot 10^6$ J surplus for growth, while known growth was only $0.658 \cdot 10^6$ J. The winter period energetics of the 1977 year-class were not modeled because of 2 of the 6 mo not having an adequate sample size.

Discussion

Predicted and observed growth agreed within 2–10% for both year-classes for the period March 15–October 15, suggesting that our assumptions and parameter estimates represent a valid application of fish energetics principles for the growing season.

During the winter, however, the balanced energy equation did not adequately describe the energetics of sauger as evidenced by a large discrepancy in predicted and observed caloric growth. We believe that there are three major areas that may contribute to this discrepancy: (1) bias related to consumption estimates, including methodology, digestion rate assumptions, and sampling, (2) growth efficiency estimates, and (3) field metabolism assumptions.

CONSUMPTION

Winter consumption by the 1977 year-class indicated that the predicted growth should have been much higher than observed. We do not believe, however, that our consumption estimates are a major source of error. Our consumption methodology is a variation of that used by Swenson and Smith (1973) and also "endorsed" by Mann (1978) in his extensive review of consumption techniques. We expanded our consumption estimates for seven sampling dates to 35 sample times by correlating feeding data taken from fish caught in 24-h net sets, with the seven daily ration estimates. This correlation was high indicating that this technique could be a useful and labor-saving approach to estimate consumption. This approach may be valid, however, only for populations which feed continously, such as predators in relatively turbid riverine and reservoir systems (Ryder 1977), such as Watts Bar (Secchi disk $\bar{x} = 94 \pm 35$ cm).

Digestion rate assumptions are another potential source of bias when estimating consumption. Laboratory digestion rate measurements spanned the range of field temperatures experienced by sauger, minimizing the bias of extrapolation to low or high temperatures. Unlike Swenson and Smith (1973), we did not adjust the digestion rate for the various meal sizes consumed by sauger because the range in meal sizes (20–60 mg/g) resulted in only a 10% variation in digestion rate estimates.

A final potential source of error involved in consumption rate estimates relates to possible sampling bias. There is some a priori reason to suspect that feeding fish are more mobile than nonfeeding fish, thus feeding fish may be selectively netted resulting in an overestimation of the population's ration. We feel that this bias is minimized by the apparent overall mobility of both the feeding and nonfeeding population. Results of paired comparison analysis between sites with low and high prey concentrations (as evidenced by gillnet catches) showed no significant differences in the percent of sauger that had fed ($P > 0.20$). If nonfeeding fish were relatively inactive and feeding fish were concentrated near high prey abundance, then significant differences in feeding between sites would be expected. Therefore, nonfeeding fish are probably also mobile and susceptible to being netted. Our gillnet catches appear, therefore, to be non-biased samples of the sauger population.

Growth Efficiency

Seasonal changes in sauger physiology is another possible source of error to account for the winter energy budget discrepancy. Although the positive relationship of metabolic energy cost and temperature dictates that growth efficiency should increase at decreasing temperatures, there is reason to suspect that in winter, physiology of sauger may be different from that represented by our energy budget equation. Such a discrepancy could result in a reduction in gross growth efficiency and, therefore, winter growth. Scale analysis indicated that no significant growth in length occurred in the winter months, as noted with other sauger and walleye populations (Hassler 1957; Kelso 1972; Kelso and Ward 1972). Energetic gains, however, occurred in fat and gonad stores. Brett et al. (1969) observed that the gross efficiency of juvenile sockeye salmon (*Oncorhynchus nerka*) was reduced at low temperatures. However, at a ration level of 60 $mg \cdot g^{-1} \cdot d^{-1}$ (the minimum ration offered to the salmon but comparable to mean rations measured for adult sauger), salmon growth over the 1−24°C experimental range was greatest at 5°C. This temperature is near the minimum experienced by Watts Bar sauger in the winter of 1979−80. In each reported case when sauger and other species have exhibited reduced growth in the laboratory during winter or at low temperatures, consumption also decreased. Thus, it is unclear if growth efficiency varied with temperature. Resolution of this question demands variable temperature growth studies with sauger at various feeding rates.

Activity Metabolism

Because our estimates of consumption during the winter appear to be valid, and growth efficiency probably changes little during the winter, there is a good possibility that discrepencies in the predicted and observed growth during the winter are due to inadequate estimates of activity metabolism. We assumed in our energy budget that field metabolism was equal to the standard metabolic rate. Winberg (1956) originally proposed that a fish's field metabolism was about twice its standard metabolic rate. Warren and Davis (1967), however, suggested that activity metabolism was a negligible portion of total field metabolism, including specific dynamic action, of a moderately active fish. Following ultrasonic telemetry studies of northern pike (*Esox lucius*) field activity, Diana (1979a, b) concluded that field metabolism of these fish approximated standard metabolism. Activity metabolism, however, can vary seasonally. Brett and Groves (1979) determined that the metabolic rate of migrating sockeye salmon was 8.5 times standard, and Adams et al. (1982) reported that the activity metabolism of largemouth bass in Watts Bar ranged from 0.18 to 1.44 times standard, depending on time of year and sex of bass.

Assuming that the discrepancy in predicted and observed growth was due almost exclusively to increased activity metabolism during the winter, an increase in field metabolism of 3−4 times standard metabolism would be required to approximate the observed growth of the 1978 year-class. Two major components of activity metabolism, foraging for food and spawning migration, would probably account for a large energy expenditure in activity during the winter. During the first part of the winter (December and January), feeding by sauger on vulnerable cold-stressed shad is intense (Fig. 2). Intense feeding results in high energy costs for foraging in other species (MacKinnon 1972; Adams et al. 1982) and probably also for sauger. During the latter part of winter (February and March), sauger migrate from the lower reaches of the reservoir to the tailwaters of the upstream dams, a distance of up to 160 km. Furthermore, sauger may remain in these areas of high current velocity for several weeks, expending energy for maintaining themselves in the currents. The period of intense feeding followed by long migration runs and residence in currents could result in activity metabolism values of 3−4 times standard and therefore may be the most plausible explanation for the discrepancies in the winter energy budget.

This study illustrates both the strengths and weaknesses of applying the energetics approach in estimating growth dynamics of fish. The close agreement of predicted and observed summer growth for two year-classes suggests that the saugers' energetics were accurately represented by the model during the growing season. This agreement is encouraging for the prospect of using the model to estimate energetics variables that are difficult to measure such as activity metabolism. The failure of the model for the winter period points out a critical lack of seasonal information. The possibility that the effect of temperature growth efficiency may be more complex than the effect of an experimental standard metabolism response (Brett et al. 1969) is a crucial but little explored phenomenon. Field activity metabolism may vary with food abundance, reproductive condition, and season. Confident application of energetics models is limited by these unknowns.

Acknowledgment

This paper is based on a M.S. thesis, University of Tennessee. We thank S. M. Bartell, J. S. Mattice, R. J. Strange, W. Van Winkle, J. L. Wilson, and especially S. M. Adams for their helpful comments on the manuscript. This research was supported jointly by the Office of Nuclear Regulatory Research, U.S. Nuclear Regulatory Commission, under Interagency Agreement DOE 40-550-75, and the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

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Reproductive Strategy of Dwarf and Normal Arctic Charr[1] (*Salvelinus alpinus*) from Vangsvatnet Lake, Western Norway

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JONSSON, B., AND K. HINDAR. 1982. Reproductive strategy of dwarf and normal Arctic charr (*Salvelinus alpinus*) from Vangsvatnet Lake, western Norway. Can. J. Fish. Aquat. Sci. 39: 1404 – 1413.

In this paper, we investigate the reproductive strategy maintaining dwarf and normal phenotypes of an Arctic charr (*Salvelinus alpinus*) population. Both morphs occurred together in the spawning area, but the peak abundance of dwarf spawners was significantly deeper, and later in the spawning period, than normal spawners. Both phenotypes attained sexual maturity at the same age and did not differ in length-specific fecundity or egg diameter. Our data support the hypothesis that the females matured sexually at the age that maximizes their fitness within the constraints imposed by growth and survival. The two female morphs appeared not to have equal fitness; these differences may be due to random events. The males were more heterogeneous by size than the females when maturing sexually. This may be due to intrasexual competition between males making large males the principal spawners. The dwarf males may circumvent the effects of intrasexual competition by mimicking immature fish, sneak at the redds of normal females, and participate in the spawning acts of normal charr pairs. Also, they may fertilize the eggs of dwarf females spawning in deeper areas.

The two charr morphs developed from a uniform young phenotype (parr). The dwarf spawners were precocious individuals maturing sexually in their parr stage, whereas normal spawners had a color shift, analogous to smoltification in anadromous charr, before they attained sexual maturity. The different spawning coloration of the two morphs may be due to sexual selection.

Key words: spawning, growth, fecundity, mortality, sexual selection, maturing strategy

JONSSON, B., AND K. HINDAR. 1982. Reproductive strategy of dwarf and normal Arctic charr (*Salvelinus alpinus*) from Vangsvatnet Lake, western Norway. Can. J. Fish. Aquat. Sci. 39: 1404 – 1413.

Dans l'article qui suit, on examine la stratégie reproductrice selon laquelle les phénotypes nains et normaux d'une population d'ombles chevaliers se maintiennent. Les deux formes sont présentes en même temps sur les frayères, mais les reproducteurs nains se trouvent en plus grande abondance à des profondeurs nettement plus grandes, et plus tard dans la saison de ponte que les reproducteurs normaux. Les deux phénotypes atteignent la maturité sexuelle au même âge, et leur fécondité ou le diamètre de leurs oeufs à une longueur donnée ne montrent pas de différences. Nos données confirment l'hypothèse que les femelles atteignent la maturité sexuelle à un âge qui porte au maximum leur bon état physique dans les limites des contraintes imposées par la croissance et la survie. Les deux formes femelles ne semblent pas être dans la même bonne condition physique, mais il se peut que les différences soient dues à des causes fortuites. Au cours de la maturation sexuelle, les mâles sont plus hétérogènes, sous le rapport de la taille, que les femelles. Ceci peut être dû à une concurrence intrasexuelle entre mâles avec, comme résultat, les grands mâles étant les principaux reproducteurs. Les mâles nains peuvent circonvenir les effets de cette concurrence intrasexuelle en imitant des poissons immatures, se glissant sur les nids de femelles normales et participant à la ponte de paires normales. Ils peuvent également féconder les oeufs de femelles naines frayant en plus grande profondeur.

Les deux formes d'ombles se développent à partir d'un jeune phénotype uniforme (tacon). Les reproducteurs nains sont des individus précoces atteignant la maturité sexuelle au stade de tacon, alors que les reproducteurs normaux subissent un changement de coloration —

[1]In this paper the spelling of the word charr differs from that (char) used in *A List of Common and Scientific Names from the United States and Canada*, by the American Fisheries Society, and is preferred by the authors.

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Printed in Canada (J6600)
Imprimé au Canada (J6600)

analogue à la smoltification d'ombles anadromes — avant d'atteindre la maturité sexuelle. La livrée nuptiale différente des deux formes peut être due à la sélection sexuelle.

Received August 21, 1981
Accepted June 19, 1982

Reçu le 21 août 1981
Accepté le 19 juin 1982

ARCTIC charr (*Salvelinus alpinus*) often feature two sympatric forms; a small form denoted dwarf charr and a large form denoted normal charr (reviews Behnke 1972; Johnson 1980). These forms may develop from one single young morph (parr, Hindar and Jonsson 1982), as has been shown in rearing experiments by Nordeng (1982). The simultaneous occurrence of both dwarf and normal spawners is apparently a paradox as there is a selective premium on large body size for both sexes. Males fight intensively in the spawning area, and one large, dominant male may fertilize the eggs of several consecutive females within the same territory (Fabricius 1953; Fabricius and Gustafson 1954). For females, the individual fecundity increases with increasing body length (Määr 1949; Jonsson and Østli 1979).

Dwarf charr are reported to spawn at greater depths and later in the breeding season than normal charr in several lakes (Määr 1949; Nordeng 1961; Svärdson 1961; Gönczi 1971; Klemetsen and Grotnes 1980). Reproductive isolation may be one reason why different forms can coexist (Kirkpatric and Selander 1979; Nyman et al. 1981), each gene pool being adapted to a specific niche (cf. table 1, Hindar and Jonsson 1982). However, Aass (1968) found that sympatric dwarf and normal charr could spawn simultaneously in the same area, making reproductive isolation unlikely. In this case, the co-occurrence of the two morphs may be explained by one of the two hypotheses: (1) the two morphs have equal fitness, or (2) they need not be equally fit, but are produced by the same genotypes. In the latter case random events may determine whether an individual develops into a dwarf or normal charr.

In this field study, we first examined the spatial and temporal distribution of charr spawners to determine whether dwarf and normal charr mated assortatively. Second, we tried to measure the fitness of dwarf and normal charr spawning at different ages by examining the growth rate, mortality, fecundity, and the age of sexual maturity. Based on these results, we investigated the reproductive strategy of the charr.

Methods

Study Area

Saganeset is the best known spawning area of the charr in Vangsvatnet Lake, western Norway (60°37′N, 6°22′E). There the charr spawn in a steep gradient at a depth from 2 to 25 m. From the depth of 2 m the substratum consists of gravel with a diameter of ~ 0.5 cm. Deeper than 5 m the substratum is gravel with some stones, 5–15 cm in diameter, intermingled. A thin layer of dy-gyttja covers the bottom from 7- to 9-m depth onwards, and it increases in thickness with increasing depth. Below a depth of ~ 20 m it is impossible to distinguish gravel and stones underneath the mud layer. A further description of Vangsvatnet Lake is given by Hindar and Jonsson (1982).

Sampling and Treatment of the Charr

A total catch of 1840 sexually immature and mature charr was sampled during the period October 1977–March 1978 in the spawning area at Saganeset and on two other localities in each of the two basins of Vangsvatnet Lake (table 2, Hindar and Jonsson 1982). An additional 1193 charr were sampled at Saganeset and in the lower basin during the autumns 1978–81.

The fish were classified as parr, dwarf, and normal charr according to their coloration (parr: immature fish with parr marks along the flanks; dwarf charr: mature fish with dull spawning colors and parr marks; immature normal charr: pink bellies and silvery sides without parr marks; mature normal charr: bright spawning colors, no parr marks; cf. fig. 4, Hindar and Jonsson 1982). We measured the natural tip length (Ricker 1979) of the charr to the nearest 0.1 cm, and we determined the sex and degree of sexual maturity according to Dahl (1917). For fecundity studies we counted ripe eggs in the ovaries of 200 females in maturity stage 4 (sensu Dahl 1917). Further, we measured the egg diameter of 10 ripe eggs of 123 females in maturity stages 5–6 to the nearest 0.1 mm. The age of the charr was determined from otoliths as described by Jonsson (1976). The otolith radius (r_1, Jonsson and Stenseth 1977), measured to the nearest 0.01 mm, gives a relative measure of the body length of the charr. The linear regression of body length (L, cm) on otolith radius (X, mm) was:

$$(1)\quad L = 20.8X - 5.11 \quad r = 0.94 \quad P < 0.001$$

The degree of overlap in the outer annulus measurements of mature and immature individuals was ≥ 80%, and thus otolith measurements could not be used with certainty to estimate the age at sexual maturity. As no other characters indicative of former breedings were found, we were unable to separate first-time and repeat breeders.

The statistical tests in the present paper are based on a 5% significance level.

Results

Reproduction

The males had a more heterogeneous length distribution than the females, among both dwarf and normal charr (Fig. 1). Also, dwarf males were on average smaller (t-test; $P < 0.005$) and normal males larger ($P < 0.001$) than the corresponding female morphs.

The first spawning (i.e. maturity stage 6) normal females were caught on November 9, whereas dwarf females did not begin spawning until 2 wk later. Normal charr males occupied the upper 15 m of the depth gradient in the spawning area from about 3 wk before spawning started until the end of the spawning period (Fig. 2). In this area the substratum seems to

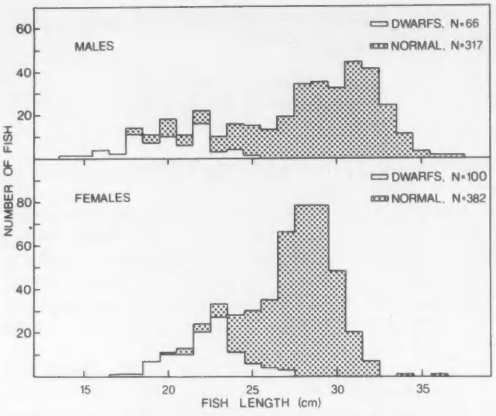

FIG. 1. Length distribution of mature male and female dwarf and normal Arctic charr (*Salvelinus alpinus*) caught with bottom nets and seine at Saganeset, Vangsvatnet Lake, during the autumns 1977–81.

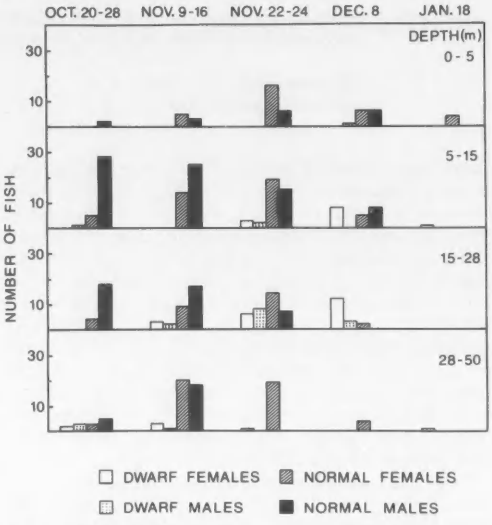

FIG. 2. Spatial distribution of mature Arctic charr (*Salvelinus alpinus*) caught with bottom nets at Saganeset, Vangsvatnet Lake, during October 20, 1977–January 18, 1978.

be most suitable for spawning (Fabricius and Gustafson 1954; Frost 1965). Dwarf males were generally found deeper than 15 m throughout the spawning season. During the first part of the spawning period the distribution of sexually mature females resembled that of the corresponding male groups; however, during late spawning (December 8) dwarf females were also found at the depth of 5–15 m. At this time, most normal females had left the spawning area and one third of those remaining were spent. The distribution of dwarf and normal charr were significantly different both with respect to depth and time (Table 1). This suggests that the morphs mainly mate assortatively, but may interbreed towards the end of the spawning season, i.e. when dwarf females and normal males were caught side by side in shallow waters. Thus, the delayed spawning of the dwarf females may be selectively favored as a result of decreased competition for spawning sites towards the end of the spawning period. The persistent dominance of normal males in shallow waters indicates that they are principal spawners throughout the spawning period. They appeared to fertilize first the eggs of normal females, then of dwarf females. The dwarf males appeared mainly to spawn deeper than 15 m, at low light intensities (Secchi disc transparency 6–7 m), and where fewer normal males were present (Fig. 2).

Deeper than 28 m most charr caught were normal females not yet ready to spawn (maturity stage 4). Most of these females must probably rise to find suitable spawning sites. This is concordant with the observation of local fishermen that the lake surface is "boiling" on the days of peak female abundance, indicating the release of excess swim bladder pressure.

Age at Sexual Maturity

The two charr morphs attained sexual maturity at the same age (Fig. 3); 50% of the charr being sexually mature at 4–5 yr of age. Most males became sexually mature at a higher age than the females (test of heterogeneity; dwarfs $\chi^2 = 12.23$, df = 5, $P < 0.05$; normals: $\chi^2 = 58.06$, df = 6, $P < 0.001$; one-tailed tests gave a significantly higher proportion of mature females than males in age-groups 4 and 5 of both morphs).

Mortality

Mortality figures from the catch curve are difficult to obtain because of irregular year-class strength (Fig. 4). According to Ricker (1969, p. 10) the best way to reduce recruitment irregularities is to combine the samples of successive years. By doing this relative numbers of charr and estimated mortality in age-groups 4–10 were obtained (Table 2). We based the calculations on the 1977–80 data, as these years appear to cover the entire period between two successive strong year-classes (cf. Fig. 4).

The calculated yearly mortality increased with age from ~ 0.15 in age-groups 4–5 to more than 0.60 in age-groups 8–10. One reason for this may be the extensive fishing for spawning charr, which accounts for about one third of the total annual catch of 6 t. The sample sizes are too small to give reliable mortality estimates for intraspecific groups of similar age (morphs and sexes). However, Fig. 3 indicates that the mortality rate of the two morphs and sexes may be similar.

Size and Growth

Normal charr were larger than parr and dwarf charr in all age-groups at the end of the growth season (Fig. 5). The differences between corresponding age and sex groups were highly significant (simultaneous *t*-tests). According to the

TABLE 1. Tests of significance (χ^2-tests) of the differences in spatial and temporal distribution of the Arctic charr (*Salvelinus alpinus*) morphs caught at Saganeset during October 20, 1977–January 18, 1978 (cf. Fig. 2). (* = $P < 0.05$, *** = $P < 0.001$.)

Dwarf charr versus normal charr				
Variable		df	χ^2	Significance
Depth	October 20–28	3	19.13	***
	November 9–16	3	7.85	*
	November 22–24	3	19.73	***
	December 8	3	23.88	***
Time		4	41.55	***

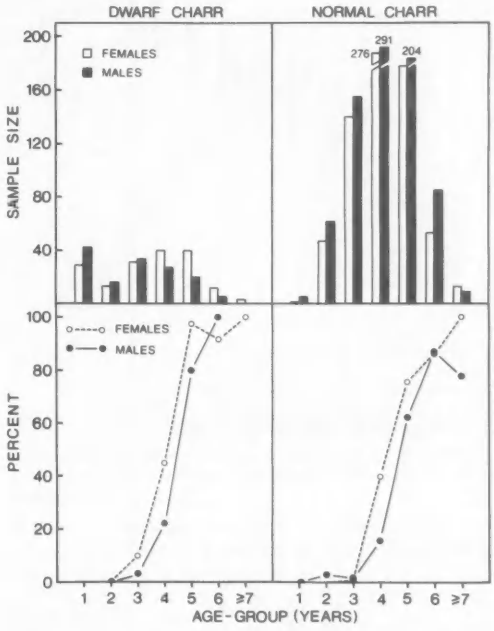

FIG. 3. Sample sizes and percentage distribution of sexually mature dwarf and normal Arctic charr (*Salvelinus alpinus*) in age-groups 1–≥7, caught in Vangsvatnet Lake during October 1977–March 1978.

Bonferroni inequality, i.e. P (global) $\leq \sum_{i=1}^{r} P_i$ (simultaneous), normal charr were overall significantly larger than dwarf charr at the 2% level.

Sexual differences in growth are reflected by the size of mature normal charr from age-group 4 onwards: the females leveled off while the males tended to grow in body length with increasing age (Fig. 5). The regression of the differences between male and female body length ($L_M - L_F$, cm) on age (A, yr) is described by the linear equation:

$$(2)\quad L_M - L_F = 0.92A - 4.11 \quad r = 0.998 \quad P < 0.001 \quad \text{for } A \geq 4 \text{ yr}$$

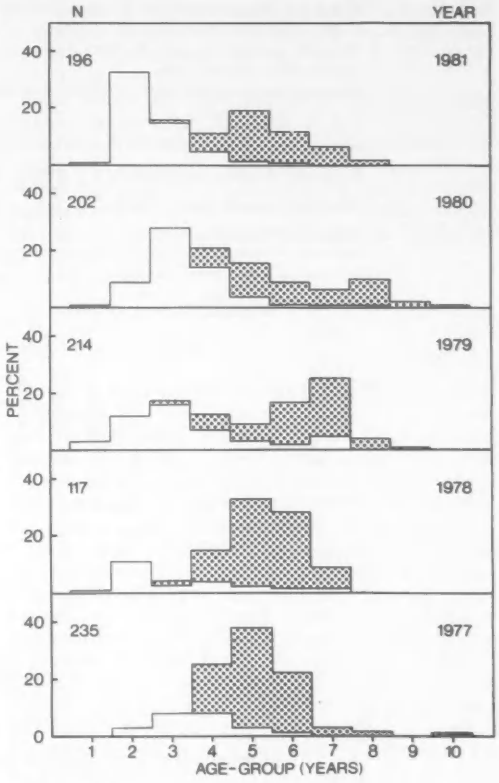

FIG. 4. Age frequency distribution of sexually immature and mature Arctic charr (*Salvelinus alpinus*) caught with bottom nets during the spawning periods (November) 1977–81. □, sexually immature charr; ⊠, sexually mature charr.

Among the dwarf charr, males were significantly smaller than females in age-groups 4 and 5 (age-group 4: $t = 2.63$, df = 41, $P < 0.025$; age-group 5: $t = 2.75$, df = 84, $P < 0.01$). Differences between the sexes are also seen when comparing the body size of immature and mature charr. These were not significantly different except for two particular cases: (1) the youngest dwarf males were significantly smaller than the corresponding parr (age-group 3: $t = 2.01$, df = 48, $P < 0.05$), and (2) the mature normal females in age-groups 4–6 were significantly larger than the corresponding immatures (age-group 4: $t = 5.59$, df = 327, $P < 0.001$; age-group 5: $t = 4.73$, df = 217, $P < 0.001$; age-group 6: $t = 1.98$, df = 126, $P = 0.05$). Both exceptions suggest sexual differences in maturing strategy.

The otolith measurements indicated that the males developing into dwarfs were the more slow-growing throughout the life span (Fig. 6). Their otolith radii were significantly smaller than even those of slow-growing (i.e. old) normal charr (simultaneous t-tests). For females, on the other hand, there were no clear-cut differences in early growth rates between

TABLE 2. Relative number of Arctic charr (*Salvelinus alpinus*) and estimated mortality in age-groups 4–10, determined from the age distribution of sexually mature charr caught at Saganeset during 1977–80, and the frequency of mature charr within each age-group as found in Vangsvatnet Lake during October 1977–March 1978.

Age-group	3	4	5	6	7	8	9	10
Average age distribution (A)	0.012	0.14	0.31	0.26	0.18	0.06	0.025	0.005
Frequency of mature individuals (F)	0.020	0.28	0.73	0.88	0.95	0.98	1.00	1.00
Relative number of charr ($1000\ A/F$)	600	500	425	296	190	61	25	5
Estimated mortality	—	0.17	0.15	0.30	0.36	0.68	0.59	0.80

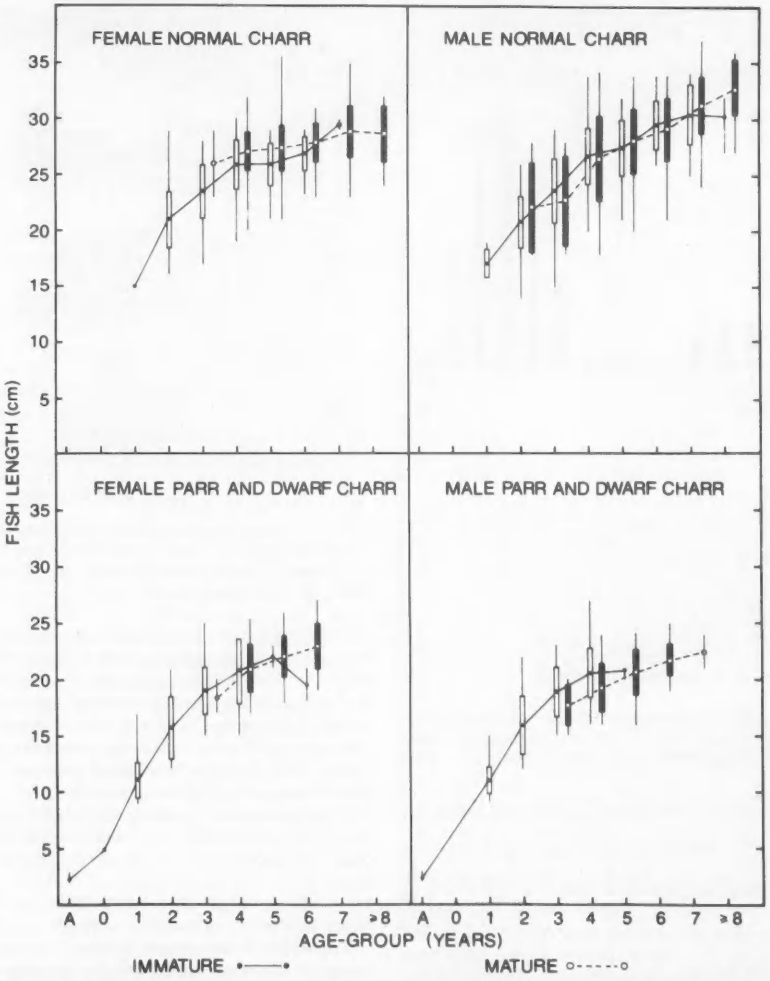

FIG. 5. Average body length, standard deviation, and total range of variance of the measurements of Arctic charr (*Salvelinus alpinus*) in the alevin stage (A) and age-groups 0–≥8, caught in Vangsvatnet Lake at the end of the growth seasons 1977–81, and on June 19–20, 1979 (alevins only). Total number of fish: 2630, standard deviation is given for $n \geq 5$.

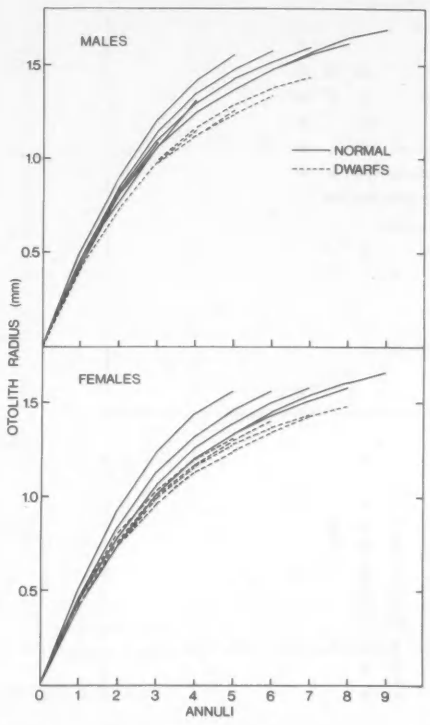

FIG. 6. Measurements of the mean radius from centrum to each hyaline winter zone (annulus) of the otoliths of sexually mature Arctic charr (*Salvelinus alpinus*) in age-groups 2−⩾8, caught in Vangsvatnet Lake at the end of the growth seasons 1977−81. Total number of fish: 1133.

dwarf and normal charr. Within the morphs, the differences in growth rate between the various age-groups appeared to be most pronounced in females.

FECUNDITY

The individual fecundity increased with body length for both charr morphs (Fig. 7). The regression of egg number (f) on female body length (L, cm) is given by the formula:

$$(3)\quad f = 51L - 717 \quad r = 0.80 \quad F = 345.6 \quad \mathrm{df} = (1,198) \quad P < 0.001$$

Covariance analysis gave no significant difference in fecundity on body length between dwarf and normal females ($F = 0.426$, $P = 0.52$).

The egg diameter (d, mm) also increased with increasing fish length (Fig. 7) and was given by the formula:

$$(4)\quad d = 0.034L + 3.23 \quad r = 0.43 \quad F = 27.31 \quad \mathrm{df} = (1,121) \quad P < 0.001$$

As with egg number, there was no significant difference in egg diameter on body length between the two morphs ($F = 2.47$, $P = 0.12$). However, the sample of dwarf charr is small and the regression coefficient for this morph alone was not significantly different from 0 ($F = 0.38$, df = (1,16), $P = 0.55$).

Older females had fewer and larger eggs than younger females of the same body length (Fig. 8). Covariance analysis gave a significant heterogeneity for both length-specific fecundity and egg diameter when using age as the breakdown variable (fecundity: $F = 3.55$, df = (3,195), $P = 0.015$; egg diameter $F = 3.27$, df = (3,118), $P = 0.024$). This result indicates that the fecundity decreases as the females become repeat breeders.

Discussion

POPULATION STRUCTURE

Although the two charr morphs largely spawned assortatively, they seem to belong to the same deme. Towards the end of the spawning season the catches suggested interbreeding between the two morphs. Furthermore, this hypothesis is supported by the observation that both morphs could be reared from eggs and sperms of the same pair of charr from Vangsvatnet Lake (H. Nordeng personal communication).

The two morphs developed from a uniform parr phenotype (fig. 4, Hindar and Jonsson 1982). For both sexes the fast-growing parr transformed to normal charr (Fig. 6). In males, only the most slow-growing parr became dwarfs, whereas in females individuals with medium growth also could develop into dwarfs. For these females the growth rate leveled off from age-group 2 onwards, indicating that the decision of whether to become dwarf or normal may be made in the late parr stage. This seems to contradict Balon's (1979, 1980) hypothesis that events in the early ontogeny determine the adult phenotype.

The dwarf charr matured while still in their parr stage and are thus homologous to the ripe parr of other salmonids, e.g. sockeye salmon (*Oncorhynchus nerka*) (Ricker 1938; McCart 1970) and brown trout (*Salmo trutta*) (Jonsson 1981). Different-sized morphs of these species may mate assortatively or may interbreed (Hanson and Smith 1967; McCart 1970; Campbell 1977; Jonsson 1981).

The spatial segregation between the morphs, especially during peak spawning, may be due to intraspecific competition. Gravel and stones as found in the shallow areas where normal charr spawned appear to be a preferred breeding substratum for charr (Fabricius and Gustafson 1954; Frost 1965). Towards the end of the spawning period, most of the normal females had left the spawning area (Fig. 2), and female competition may be more lax at that time. Normal males stayed longer in the spawning area and displaced most dwarf males from shallow areas throughout the spawning period.

SEXUAL SELECTION

The nuptial dress of the charr may be related to their competitive ability. Fabricius and Gustafson (1954) found that sexually mature charr were aggressive against red objects. Apparently then, a red belly color is disadvantageous as it

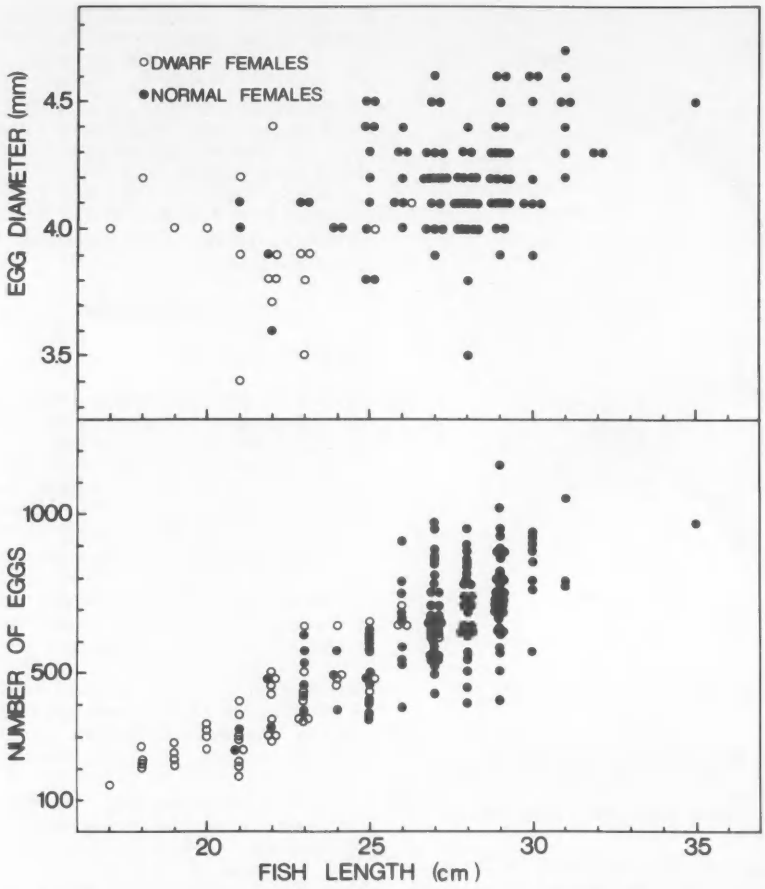

FIG. 7. Egg diameter and number of eggs per female in Arctic charr (*Salvelinus alpinus*) caught in Vangsvatnet Lake during the autumns 1977–81.

triggers aggressive behavior towards the carrier. Only large males may be strong enough to flaunt bright red spawning colors and still defend a territory, and their nuptial dress may thus be a signal to the females of a competitive mate holding a good spawning site (cf. Fabricius and Gustafson 1954). This sexual selection (Darwin 1888; Maynard Smith 1978) gives an evolutionary explanation of why small males are paler than large males, and why males in general are more brightly colored than females of corresponding body lengths (fig. 4, Hindar and Jonsson 1982). Intersexual aggression (Fabricius and Gustafson 1954) may be reduced by the black pigment along the sides of the female bellies; the females may thus not show their belly colors when resting on the bottom.

Intrasexual aggression may be reduced by the similar coloration of dwarf spawners and parr. The parr marks of the dwarf spawners indicate that the dwarfs mimic immature fish (parr) in coloration, possibly to avoid the aggression of normal spawners. Therefore, small males may be able to sneak in and fertilize a part of the eggs of females principally spawning with large males. Their depth distribution suggests that sneaking may be favorable at low light intensities. Sneaking may be difficult in more shallow areas, because of the lighter environment and the higher density of the normal morph.

Among river spawners the proportion of dwarf males may be high (Gritsenko 1971). In running water sneaking may be favorable because of decreased visibility due to the water current. Furthermore, rivers often offer shallow areas in the vicinity of the redds where large fish are unable to chase small ones because of their body size difference. Thus, the ratio of dwarf versus normal males may vary in different habitats as a result of differences in reproductive success of the two morphs.

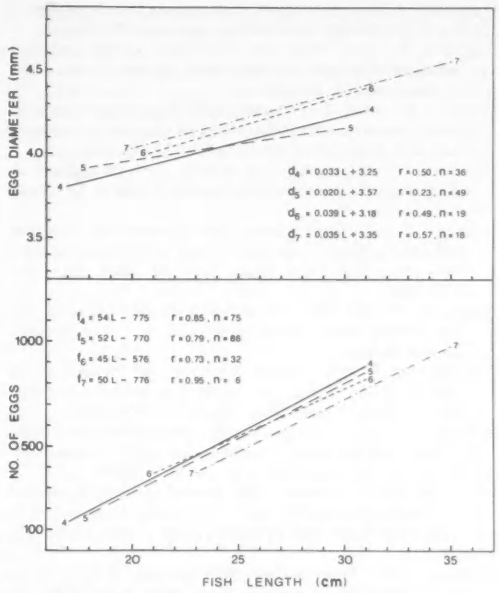

FIG. 8. Regression of egg diameter and number of eggs per female on body length of Arctic charr (*Salvelinus alpinus*) in age-groups ≤4−≥7 caught in Vangsvatnet Lake during the autumns 1977−81.

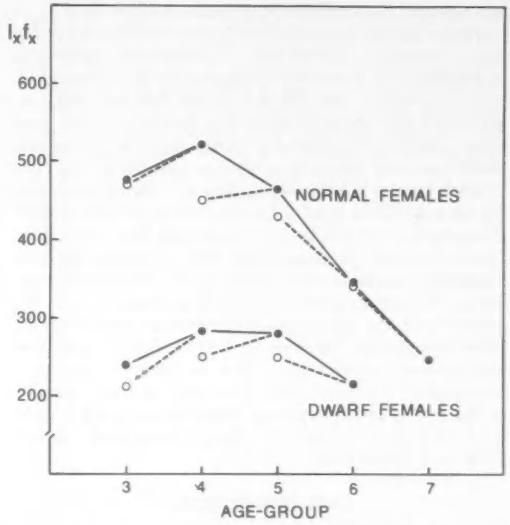

FIG. 9. Age-specific net reproductive rate ($l_x f_x$) as a function of age (x); shown for age-groups 3−7 (●), and for the same cohorts 1 year earlier (○). The age-specific survival rate is designated l_x, the age-specific fecundity f_x. The parameters are estimated from data given in Table 2, Fig. 5 and 6, and Eq. (1) and (3).

MATURING STRATEGY

In a recent paper Jonsson (1981) hypothesized that within an equilibrium population (ESS) the age of sexual maturity for females largely depends on the age-specific survival rate (l_x) and fecundity (f_x) of the fish, i.e. that the selective premium is highest for females maturing sexually at the age (a) when

$$(5)\quad \sum_{x=a}^{b} l_x f_x > \sum_{x=a}^{b} l_{x+1} f_{x+1}$$

where b designates the age at death.

These terms may be evaluated by comparing the corresponding $l_x f_x$ values; the reason for doing this approximation being that mortality appears to be more influenced by sexual maturity and spawning than by the physiological age. The age-specific survival (l_x) of the morphs and age-groups may be calculated from the survival data (Table 2), and the age-specific fecundity (f_x) from the mean female length of each age-group (Jonsson 1977). The results indicate that normal females (except for age-group 7) have higher fitness than dwarf females (Fig. 9). Furthermore, it appears to be most favorable for normal females to mature in age-group 4, and for dwarf females to mature in age-groups 4 and 5. Within each morph the $l_x f_x$ values of age-group 5 and older are probably underestimated relative to younger fish, as these fish have reduced survival and growth due to the high proportion of already mature charr. Both dwarf and normal charr of age-groups 4 and 5 had higher $l_x f_x$ values than these cohorts had the previous year. This suggests that the various morphs and age-groups may have different fitness, but still maximize their $l_x f_x$ values within the constraints imposed by experienced growth, and future expected growth and survival. Age-groups 6 and 7 had lower $l_x f_x$ values compared with the previous year, which indicates that most of these fish should already be sexually mature. Unfortunately, we are unable to distinguish between first-time and repeat spawners.

The present model (5) assumes that survival and growth of the progeny are independent of the size of their mother, and that differences within the stock are largely due to growth differences, not to genetics. To be an ESS strategy (Maynard Smith 1976), the splitting of the young parr into different cohorts must be due to random events, e.g. that different individuals happen to find different amounts of food, and thereby experience different growth rates (Alm 1959). This is in accordance with Nordeng's (1982) experiments, showing a low heritability of the dwarf character. What is inherited is apparently a growth-dependent maturity pattern, giving the proper reaction (i.e. smoltification, sexual maturity, or not) to a given stimulus (i.e. growth rate). As the survival rate may vary between locations and years, one may expect a certain variation in how individual females respond to growth with respect to smolting and sexual maturity. Hitherto, however, little genetic variation has been found within Arctic charr populations (Ferguson 1981; Ryman and Ståhl 1981).

The alternative hypothesis, i.e. that dwarf and normal females have equal fitness, seems unrealistic, as the survival rate of dwarf females from birth to maturity must be twice that of normal females to compensate for their lower fecundity (Fig. 9). Thus, our data appear to support the hypothesis that the two morphs belong to the same genotypes, all individuals

having equal expected fitness at birth.

The model (5) proposed for females may also apply for males. However, male fecundity is difficult to quantify as it is determined by the number of eggs fertilized in competition with other males. Two different tactics, fight or sneak, are possible. Large dominant males have been found to be principal spawners (Fabricius and Gustafson 1954). However, small males may also be fit as they can both fertilize the eggs of small females in deep areas, and sneak in during the spawning act and fertilize a part of the eggs of large females primarily spawning with a large male (Jones and King 1952). The length distribution of mature males (Fig. 1) suggests that both small dwarf and large normal males may be successful spawners in Vangsvatnet Lake. The growth rate appears to determine whether the sneaker or the fighter tactic is the better for an individual male. The growth patterns in Fig. 6 suggest that slow-growing individuals do best as sneakers, and fast-growing individuals as fighters. Thus, both males and females of the two charr morphs may mature sexually when their expected fitness is maximized within the constraints imposed by growth and survival.

Acknowledgments

Our sincere thanks go to Finn R. Gravem, Ørnulf Haraldstad, Dag Matzow, and Tormod A. Schei for excellent assistance and support throughout this work. We also thank Erik Bleken, Mart R. Gross, Thomas Johnston, Hans Nordeng, and Jens Petter Nilssen for valuable discussions, and the Journal assistant editor for constructive criticism of the manuscript. In addition, we thank Knut Kvalvågnæs and Bjørn Rørslett for photographing the spawning-area substrates, Ingeborg Gjøen for drawing figures, Liss Fusdahl and Ragnhild Frilseth for typewriting the manuscript, and Veronica Phillips Olsen for improving the English. At Voss, we thank Johan Arnøy and Jørn Skjerven for helpful cooperation. The Municipality of Voss and the Zoological Institute of the University of Oslo provided material and financial support. Furthermore we acknowledge financial support from the Faculty of Science, University of Oslo; the Norwegian Research Council for Science and the Humanities; the Norwegian Directorate for Wildlife and Freshwater Fishes; and the International Symposium on Arctic Charr, Winnipeg. This is a contribution from the Voss Project, Zoological Institute, University of Oslo.

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Homing by Canada's West Coast Herring to Management Units and Divisions as Indicated by Tag Recoveries

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HOURSTON, A. S. 1982. Homing by Canada's west coast herring to management units and divisions as indicated by tag recoveries. Can. J. Fish. Aquat. Sci. 39: 1414 – 1422.

The change from a fishery for reduction to meal and oil to one primarily for roe resulted in major shifts in the herring fishing grounds on Canada's west coast. To meet management needs for the new fishing pattern, the former stock concept of seven "populations" was revised into 35 "management units." Reanalysis of tag recovery data for the period 1937–67, in terms of the 22 management units represented, showed a degree of homing between 66 and 96% for 18 of these management units. This is comparable to the homing displayed by the seven former populations (54–84%) estimated from essentially the same data base. Homing to some of the more isolated management units was comparable to that for the six major divisions of the coast (77–94%), as currently defined. Stevenson's (1954) conclusion "... the herring of British Columbia tend to form a series of integrating populations" is supported by the present analysis.

Key words: *Clupea harengus pallasi*, tagging, homing, stocks, populations

HOURSTON, A. S. 1982. Homing by Canada's west coast herring to management units and divisions as indicated by tag recoveries. Can. J. Fish. Aquat. Sci. 39: 1414 – 1422.

À la suite du changement d'une pêche pour la transformation en farine et en huile de poisson en une pêche visant surtout la rogue, il se produisit d'importants déplacements des lieux de pêche du hareng sur la côte ouest du Canada. Afin de satisfaire aux besoins de gestion créés par cette nouvelle répartition de la pêche, le concept de sept « populations » de jadis a été révisé et remplacé par 35 « unités de gestion ». Après réexamen des données sur les recaptures d'étiquettes en 1937–67 en relation avec les 22 unités de gestion représentées, on a constaté un homing de 66 à 96% dans 18 de ces unités. Ceci se compare avec le homing exhibé par les sept populations antérieures (54–84%) estimé à partir essentiellement des mêmes données. Le homing vers quelques-unes des unités de gestion plus isolées est comparable à celui des six principales divisions de la côte (77–94%), telles que présentement définies. La présente analyse confirme la conclusion de Stevenson (1954) « ... le hareng de la Colombie-Britannique tend à former une série de populations intégrantes ».

Received November 27, 1981
Accepted May 26, 1982

Reçu le 27 novembre 1981
Accepté le 26 mai 1982

POPULATIONS of Pacific herring (*Clupea harengus pallasi*) on Canada's west coast (Fig. 1) were originally identified on the basis of mean vertebral number (Tester 1937, 1949). Since the mid 1940s, both the number of populations and their geographic boundaries have been modified from time to time (e.g. Stevenson 1954, 1955; Taylor et al. 1956; Taylor 1964) on the basis of 36 264 tag recoveries from fish tagged on the spawning grounds between 1937 and 1967 (Hourston 1981a). These recoveries came exclusively from commercial catches by a reduction fishery. This fishery operated most effectively on migrating fish en route to their spawning grounds, when the fish were concentrated in relatively sheltered waters close to the processing plants. However, because much of the value of the product came from the oil produced, and because the oil content of the fish decreased rapidly during the final stages of maturation, the fisheries tended to operate as early in the migration as was feasible without foregoing the above-mentioned advantages. Consequently, the smallest stock unit relevant to management of the fisheries (i.e. the fish supporting the catch on a single fishing ground) was frequently made up of a mixture of smaller stock units which subsequently became segregated as they moved on to their separate spawning grounds. "Stock unit" is used herein as a general term for an aggregate of organisms which is in some sense distinct from other aggregates of the same species. Thus, the "populations" referred to above and the "management units" and "divisions" introduced below represent differently defined concepts of stock units.

With the advent in the early 1970s of processing primarily for ripe roe, fishing activity moved from a few major migration routes to a relatively large number of major spawning grounds (Hourston and Haegele 1980). It quickly became evident that managing the fisheries on the basis of the large

Printed in Canada (J6735)
Imprimé au Canada (J6735)

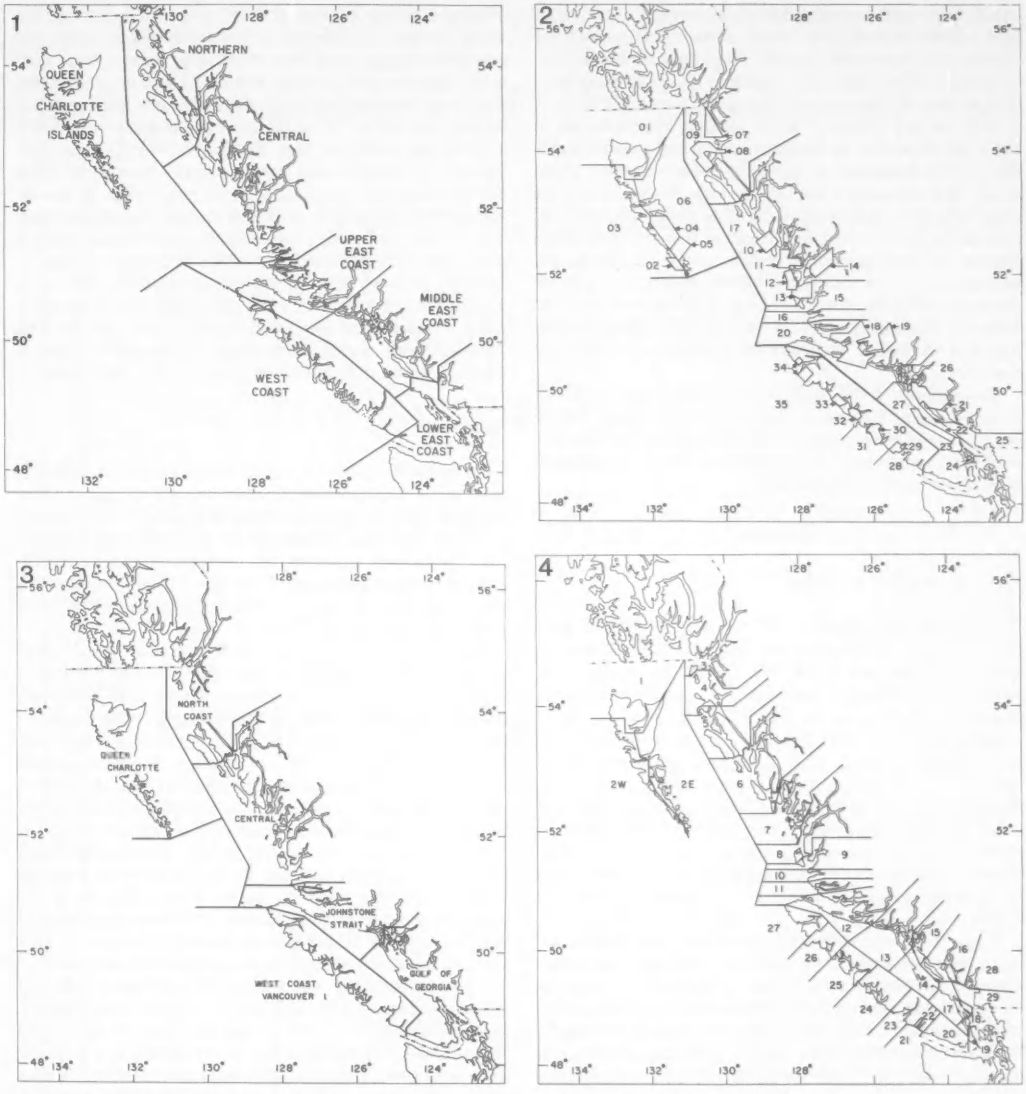

FIG. 1. Populations for the former reduction herring fishery in British Columbia. FIG. 2. Management units for the current roe herring fishery in British Columbia. FIG. 3. Divisions for the current roe herring fishery in British Columbia. FIG. 4. Statistical areas in British Columbia.

geographic units occupied by populations as previously defined could lead to the depletion or even elimination of runs to some spawning grounds, while allowing an excess of spawners on others. On this basis, 25 "management units" (geographic regions supporting or with potential for a roe fishery and including the spawning grounds utilized by these fish) were identified (Hourston and Hamer 1979). Other sectors of the coast excluded from these 25 management units were assigned to one of 10 additional management units on the basis of geographic proximity to provide a managerial format for assessing any past or future appearances of herring in these regions.

The extent to which herring spawning in each of these management units (Fig. 2) "home" to the same management

unit in subsequent spawning migrations provides a measure of their validity as biological stocks. Tester (1949) considered 75% or more homing as the basis for population delineation. Stevenson (1954) found that homing to the seven major populations then defined ranged between 85.6 and 55.5%.

With the introduction of "area" licensing into the fishery in 1981, the need arose for the most accurate information available on the abundance of herring in broader regions of the coast. This information was provided for six divisions of the coast (Fig. 3). Four of these divisions parallel four of the populations used during the reduction fishery, with minor changes in boundaries. The middle east coast population, whose integrity has now become questionable, was split between the upper and lower east coast populations to form the Johnstone Strait and Gulf of Georgia divisions. Thus divisions represent an updated version of the populations as originally described.

The purpose of this study is to reanalyze the results of 30 yr of herring tagging and tag recovery on Canada's west coast to assess the probable extent of homing to the management units and divisions which form the basis of the management of the current roe fisheries.

Methods

Tagging and Tag Recovery

Procedures for tagging and tag recovery have been described in detail many times in connection with the original study (e.g. Hart and Tester 1937, 1938; Stevenson 1954; Taylor 1964). These procedures are summarized briefly below.

Coded metal tags were inserted in the body cavity of herring captured by seine. Most of the taggings of adults were carried out on spawning grounds spread among 28 of the 35 management units on the coast (Table 1, Fig. 2). Only taggings on spawning grounds were included in the present study. Juveniles were tagged with similar but smaller tags close to where they were spawned before they had an opportunity to mix with juveniles from other spawnings (Hourston 1959).

Tagged fish were recovered as they were unloaded at the major reduction plants using electronic metal detectors which tripped a trapdoor which deflected a group of fish, including the tagged one, into a bin for subsequent examination. Such recoveries could readily be traced to the vessel delivering the fish and hence the location of capture of the tagged fish could usually be definitely established. Tags which escaped detection by this means, or passed through plants without detectors, were recovered during processing by magnets in the meal lines. The location of capture of the fish carrying these tags could only be traced to the locations of capture of fish processed by that plant during this time period. Some of the smaller plants processed only locally caught fish so tag recoveries from these plants could be assigned with confidence to area of capture. However, most magnet recoveries could only be attributed to several possible areas of capture.

The efficiency of detectors and magnets in the various plants in recovering tags and the time lag to recovery at the various magnets were tested periodically during the season by placing a known number of "test" tags in fish entering the plant. The results of these tests, along with data on catches not processed through meal lines containing magnets, were used to estimate the number of tags that would have been recovered each year from each area had all of the catch been searched for tags and had all of the detectors and magnets been 100% efficient at recovering tags (Hourston 1981a). These "adjusted" recoveries should provide a better quantitative basis for assessing the distribution of tag recoveries. However, because the information on which the adjustments are made was sometimes lacking or inadequate, and because adjusted recoveries were estimated from either detector or magnet recoveries (but not both) to the exclusion of recovery areas included in one but not the other, these data may be biased in some instances. The data were compiled, therefore, for both "adjusted" and "actual" recoveries so that anomalies could be checked for the effects of such bias on either set of data.

Analysis

To assess the extent of homing to the roe fishery management units, it is necessary to array existent tagging and tag recovery data in a form as relevant as possible to this concept of stocks. Because tagging data have been recorded historically by locality and date, this information can be readily arrayed by management unit (Hourston 1981a). The situation with respect to tag recovery data is, however, much more complex.

Tag recovery data were originally recorded by statistical area (Fig. 4) and summarized by population (e.g. Stevenson et al. 1952). In some years, towards the end of the program, recovery location records for individual tags were limited to the seven major populations (Fig. 1) as defined by Taylor and Outram (1954), and hence to the group of statistical areas included in each population. Consequently, the closest that recovery data could be related to management units was in the form of statistical areas or combinations thereof (Hourston 1981a). As a result, estimates of homing indicated the return to that management unit and to the immediately adjacent waters within that statistical area(s). While this procedure would tend to overestimate homing to the management unit, in most instances any other stocks involved were exceedingly small in relation to the run to the management unit itself.

To circumvent any bias resulting from the geographically spotty coverage of detector searches for tags, it was assumed that all detector recoveries would have been picked up by magnets had they not been previously removed, and recoveries from both of these sources were combined. Recoveries of the smaller juvenile tags, which were applied in the fall as these fish left their rearing areas for offshore feeding areas (Hourston 1959), were compiled separately.

The percent homing to each management unit was estimated in three ways: (1) certain returns [recoveries known to have come from the statistical area(s) involved] as a percentage of all recoveries; (2) certain returns as a percentage of certain recoveries (excluding recoveries that could have come from either inside or outside the homing area); (3) possible returns (recoveries which came from the homing area or any combination of areas including the homing area) as a percentage of all recoveries. The first approach gives a minimum

TABLE 1. Percent homing to management units as indicated by (1) certain returns/all recoveries, (2) certain returns/certain recoveries, (3) possible returns/all recoveries. Data for actual recoveries are given on the first line, followed by data for adjusted recoveries on the next line. Data for juvenile tags are marked with asterisks. Alternative data are given when mixing with adjacent management units was extensive.

		Method								
		(1)			(2)			(3)		
Management unit	Recovery area	Certain returns	All recoveries	%	Certain returns	Certain recoveries	%	Possible returns	All recoveries	%
02 Louscoone Inlet	2BW	0	153	0	0	153	0	0	153	0
		0	199	0	0	199	0	0	199	0
	2BW, 2BE	139	153	90.8	139	153	90.8	139	153	90.8
		179	199	90.0	179	199	90.0	179	199	90.0
03 Other Area 2W	2AW	286	330	86.7	286	330	86.7	286	330	86.7
		439	503	87.3	439	503	87.3	439	503	87.3
05 Skincuttle Inlet	2BE	273	928	29.4	273	343	79.6	858	928	92.5
		357	1303.1	27.4	357	455.6	78.4	1204.5	1303.1	92.4
06 Other Area 2E	2AE, 2BE	248	1121	22.1	248	458	54.2	911	1121	81.2
		330	1536.8	21.5	330	623	53.0	1243.8	1536.8	80.9
07 Chatham Sound	3, 4	221	552	40.0	221	296	74.7	447	552	81.0
		221	670.6	33.0	221	346.8	63.7	544.8	670.6	81.2
08 Porcher Island	4, 5	84	2038	4.1	84	588	14.3	1534	2038	75.3
		739.2	2312.4	32.0	739.2	966	76.5	2085.6	2312.4	90.2
10 Kitasu Bay	6	148	1672	8.9	148	356	41.6	1464	1672	87.6
		1353.2	1886.8	71.7	1353.2	1663.7	81.3	1576.4	1886.8	83.6
11 Milbanke Sound	7	393	2318	17.0	393	927	42.4	1784	2318	77.0
		1111.2	2545.5	43.7	1111.2	1686.8	65.9	1969.9	2545.5	77.4
12 Queens Sound	7	36	1123	3.2	36	89	40.4	1070	1123	95.3
		802.2	1434.1	55.9	802.2	837.5	95.8	1398.8	1434.1	97.5
13 Kwakshua Channel	8	151	981	15.4	151	188	80.3	944	981	96.2
		151	1073.7	14.1	151	508.7	29.7	716	1073.7	66.6
15 Rivers Inlet	9	1	20	5.0	1	9	11.1	12	20	60.0
		1	15	6.7	1	4	25.0	12	15	80.0
16 Smith Inlet	10	2	179	1.1	2	82	2.4	99	179	55.3
		0	256.2	0	0	246.6	0	96	256.2	37.5
18 Upper Johnstone St.	12	461	1078	42.8	461	871	52.9	668	1078	62.0
		1050.4	1738.7	60.4	1050.4	1520.5	69.1	1268.6	1738.7	73.0
20 Other Johnstone St.	12, 13	96	338	28.4	96	148	64.9	286	338	84.6
		304.7	582.7	52.3	304.7	369.7	82.4	517.7	582.7	88.9
21 Powell River	15, 16	1	869	0.1	1	284	0.35	586	869	67.4
		0*	1*	0*	0*	0*	0*	1*	1*	100*
		1	1227.1	0	1	636.9	0.2	591.2	1227.1	48.2
		0*	1*	0*	0*	0*	0*	1*	1*	100*

TABLE 1 (*Concluded*)

Management unit	Recovery area	Method (1) Certain returns	(1) All recoveries	(1) %	Method (2) Certain returns	(2) Certain recoveries	(2) %	Method (3) Possible returns	(3) All recoveries	(3) %
22 Nanaimo–Comox	14, 17	100	1538	6.5	100	452	22.1	1186	1538	77.1
		562.1	2484.5	22.6	562.1	1351.9	41.6	1694.7	2484.5	68.2
	14, 17, 18	406	1538	26.4	406	452	89.8	1494	1538	97.1
		1051.8	2484.5	42.3	1051.8	1351.9	77.8	2186.4	2484.5	88.0
23 Yellow Point	17	48	660	7.3	48	301	16.0	407	660	61.7
		0*	1*	0*	0*	0*	0*	1*	1*	100*
		282.3	1165.2	24.2	282.3	796.9	35.4	650.6	1165.2	55.8
		0*	1.2*	0*	0*	0*	0*	1.2*	1.2*	100*
	17, 18	224	660	33.9	224	301	74.4	583	660	88.3
		439.5	1165.2	37.7	439.5	796.9	55.2	807.8	1165.2	69.3
24 Ganges–Plumper	18	92	431	21.4	92	199	46.2	324	431	75.2
		230.9	692.5	33.3	230.9	542.2	42.6	381.2	692.5	55.1
28 Other Area 23	23	492	1189	41.4	492	601	81.9	1080	1189	90.8
		11*	40*	27.5*	11*	26*	42.3*	25*	40*	62.5*
		460	1109.2	41.5	460	572.6	80.3	996.6	1109.2	89.9
		16*	61.6*	26.0*	16*	41.7*	38.4*	35.9*	61.6*	58.3*
29 West Barkley	23	1087	2776	39.2	1087	1410	77.1	2453	2776	88.4
		0*	1*	0*	0*	1*	0*	0*	1*	0*
		3071	4175.6	73.6	3071	3665.8	83.8	3580.8	4185.6	85.8
		0*	1.8*	0*	0*	0*	0*	0*	1.8*	0*
30 South Clayoquot	24	51	759	6.7	51	242	21.1	568	759	74.8
		71	970.9	7.3	71	594.4	11.9	447.5	970.9	46.1
31 Sydney Inlet[a]	24	13	927	1.4	13	492	2.6	448	927	48.3
		1*	10*	10.0*	1*	7*	14.3*	4*	10*	40.0*
		13	1394.3	0.9	13	1183.4	1.1	223.9	1394.3	16.1
		1.7*	17*	10.0*	1.7*	14.2*	12.0*	4.5*	17*	26.5*
	24, 25	211	927	22.8	211	479	44.1	669	927	72.2
		608	1394.3	43.6	608	1170.4	52.0	827.3	1394.4	59.3
32 Nootka Sound	25	282	1197	23.6	282	483	58.4	996	1197	83.2
		955.4	1896.4	50.4	955.4	1429.7	66.8	1422.1	1896.4	75.0
33 Nuchatlitz Inlet	25	381	1075	35.4	381	457	78.2	999	1075	92.9
		1218.6	1826.7	66.7	1218.6	1356.7	89.8	1688.6	1826.7	92.4
34 Quatsino Sound	27	208	770	27.0	208	585	35.6	393	770	51.0
		209	936.3	22.3	209	659	31.7	486.3	936.3	51.9
35 Kyuquot Sound[b]	26	127	1377	9.2	127	615	20.7	889	1377	64.6
		127	3387.9	3.8	127	1897.2	6.7	1617.7	3387.9	47.8

[a]Part of Other Area 24 management unit (Hourston and Hamer 1979).
[b]Part of Other Upper West Coast management unit (Hourston and Hamer 1979).

estimate of homing, for it assumes that all recoveries which could have come from outside the homing area did so. The second approach gives a more realistic estimate of homing in that it excludes all recoveries which could have been taken either inside or outside the homing area. It has, however, the disadvantage of excluding about half of the recovery data. The third approach gives a maximum estimate of homing in that it assumes that all recoveries which could have come from inside the homing area did so.

Results

Homing by Management Unit

Of the 12 management units which have provided most of the roe catch in recent years, seven (Other Area 2W, Skincuttle, Porcher, Kitasu, Queens, West Barkley, and Nuchatlitz) showed homing (Table 1) of 75% or more (Tester's 1949 criterion for population differentiation) on the basis of adjusted certain recoveries (method 2). Two of the remainder (Louscoone, Nanaimo–Comox) were harvested by the reduction fishery while migrating through an adjacent area: when this area was included in the homing area, homing exceeded 75% for these two management units also. Two other management units (Milbanke and Nootka) showed homing approaching this level (66 and 67%, respectively). All of these management units showed a potential homing (method 3) of over 75%, and all but two (Skincuttle 27% and Porcher 32%) showed a minimum homing (method 1) of over 40%. Thus, on the basis of the same tag recovery data, these 11 management units appear to display a discreteness comparable to the seven populations analyzed by Stevenson (1954) for which homing ranged from 56 to 86%.

The South Clayoquot homing estimate was based on only 51 certain recoveries. Reduction catches from this area contained much smaller proportions and numbers of fish of age IV and older (repeat spawners) than did the catches from adjacent areas (Hourston et al. 1972), providing little opportunity for recovery in the home area of tags inserted on fish which had spawned in previous years. Moreover, there were no reduction plants in Clayoquot Sound; consequently catches from that area had to be packed to Barkley Sound or Nootka Sound, where they would have become mixed with local catches. This severely limited the possibilities for certain recoveries from Area 24. On this basis, the possible returns (all tags which could have been recovered in Area 24, method 3) might be considered better to represent homing to the South Clayoquot management unit. Moreover, this is one of only three management units for which the adjusted recoveries were appreciably fewer than the actual recoveries, making the former suspect. This leaves possible actual returns as the best available measure of homing to the South Clayoquot management unit (75%) which would place it within the range of a separate stock as defined above.

Of the other 14 management units in which tagging was conducted, 4 showed homing comparable to the major management units (Chatham, 64%; Upper Johnstone, 69%; Other Johnstone, 82%; and Other Area 23–80%). Two others appear to have distorted values for adjusted recoveries but show strong homing from actual recoveries (Kwakshua, 80%; Yellow Point, 74%). Four others produced too few actual recoveries in the home area to permit meaningful estimates of homing (Rivers, 2; Smith, 2; Powell River, 1; Sydney Inlet, 13). The four remaining management units (Other Area 2E, Ganges–Plumper, Kyuquot, and Quatsino) failed to show significant homing by any of the criteria examined.

In summary, there were sufficient tag recoveries from the home area to assess the degree of homing for 22 of 35 management units. Of these, 15 (including 11 of the 12 major suppliers of the roe fishery) showed homing comparable to that of the seven major populations assessed by Stevenson (1954) from the same data, and 3 others (including the other major roe fishery) are considered likely to have done so. Only four minor management units failed to exhibit a significant degree of homing.

Homing by Division

As anticipated, homing to the larger geographic units represented by the six divisions was higher than for most management units, ranging between 77 and 94% based on adjusted certain returns (Table 2, method 2). However, the fact that half of the 22 management units exhibited homing within this range or higher (Table 1) suggests that smaller geographic units have equal validity as stocks on the basis of tag recovery data. Four other management units exhibited homing close to this level (64–74%) and two others could be included on the basis of actual returns. Thus over three-quarters of the 22 management units involved exhibited homing comparable to, or close to, that for the six divisions. Pending reassessment of the status of the other five management units, it would appear that the management unit concept of stock units, which was developed in response to needs identified for the management of roe fisheries under current fishing practices, is of comparable validity to the population concept of stock units used to manage the reduction fishery in the 1940s to 1960s, and close to that for the broader stock concept of divisions.

Except for the lower values for method 1 (certain returns/all recoveries) for the Johnstone Strait and Gulf of Georgia divisions, the estimates of homing to division are remarkably consistent between methods and for actual and adjusted recoveries. This supports the approach of selecting the most appropriate data base for each stock which was utilized in the analysis by management units.

Homing by division was somewhat higher than that estimated by Stevenson (1954) for seven major populations. This may be attributed to the enlargement of the data base since 1954 and the larger geographic area of most divisions compared to their population counterparts.

Homing by Juveniles

Tagging of juveniles was concentrated on fish hatched and reared in the Other Area 23 management unit. As there was no tagging elsewhere in Area 23, there is little to add in the present context to the conclusions reported previously (Hourston 1959) that tagged juveniles express a lower degree of homing than do tagged adults at large for the same number of years.

TABLE 2. Percent homing to major herring divisions in British Columbia as indicated by: (1) certain returns/all recoveries; (2) certain returns/certain recoveries; possible returns/all recoveries. Data for actual recoveries are given on the first line, followed by data for adjusted recoveries on the next line. Data for juvenile tags are marked with asterisks.

	Method								
	(1)			(2)			(3)		
Division	Certain returns	All recoveries	%	Certain returns	Certain recoveries	%	Possible returns	All recoveries	%
Queen Charlotte Islands	2345	2552	92	2345	2547	92	2350	2552	92
	3311.3	3565.9	93	3311.3	3559.9	93	3317.6	3565.9	93
North Coast	2005	2425	83	2005	2397	84	2033	2425	84
	2971.2	3456.7	86	2971.2	3442.8	86	2985.1	3456.7	86
Central	7489	8311	90	7489	8007	94	7797	8311	94
	8718.3	9248.7	94	8718.3	9236.7	94	8730.3	9248.7	94
Johnstone Strait	962	1709	56	962	1141	84	1530	1709	90
	1645.7	2664.8	62	1645.7	2036.6	81	2273.9	2664.8	85
Gulf of Georgia	1923	4434	43	1923	2447	79	3910	4434	88
	6*	8*	75*	6*	8*	75*	6*	8*	75*
	3688.9	6898.3	53	3688.9	4811.8	77	5775.4	6898.3	84
	7.1*	9.5*	75*	7.1*	9.5*	75*	7.1*	9.5*	75*
West Coast Vancouver Island	10558	11300	93	10558	11295	93	10563	11300	94
	19*	51*	37*	19*	34*	56*	36*	51*	71*
	16072.0	17167.8	94	16072.0	17162.8	94	16077.0	17167.8	94
	28.8*	80.4*	36*	28.8*	59.5*	48*	49.7*	80.4*	62*
All	25282	30731	82	25282	27834	91	28183	30731	92
	25*	59*	42*	25*	42*	60*	42*	59*	71*
	36407.4	43002.2	85	36407.4	40250.6	90	39159	43002.2	91
	34.9*	89.9*	40*	35.9*	69.0*	52*	56.8*	89.9*	63*

YEARS AT LARGE

Homing to the various management units varied considerably according to the number of years the tagged fish were at large (Hourston 1982). This applied to estimates from both actual and adjusted recoveries. The degree of homing showed no consistent trends in relation to the number of years at large, suggesting that conditions of tagging and/or environmental conditions during the prespawning migration in the year of recovery have a greater effect on the degree of homing than the number of years a tagged fish was at large.

Discussion

Stevenson (1954) concluded, on the basis of homing to the seven major herring populations then defined for the British Columbia coast, that "... the herring in British Columbia tend to form a series of integrating populations." Our reanalysis of the expanded (to 1967) data base for a larger number (26) of geographically smaller management units supports that conclusion. While taggings in all management units which yielded more than one recovery showed some straying, highly isolated major management units, such as Other Area 2W, Nanaimo–Comox, West Barkley, and Nuchatlitz showed a much higher degree of homing (87, 90, 84, and 90%, respectively) than did major management units with other management units located on either side, such as Milbanke Sound, Nootka, and South Clayoquot (66, 67, and 75%, respectively). Furthermore, a comparison of recoveries of tags inserted in juvenile and adult fish which had been at large for the same number of years suggested that homing was related more to previous spawning experience than to where the fish themselves hatched (Hourston 1959). This too is consistent with the concept of "... a series of integrating populations" with appreciable intermixing, particularly with neighboring spawning stocks.

On this basis, it would be anticipated that dividing the coast into regions at areas of discontinuity in the distribution of spawning grounds would serve to define a series of herring stocks which demonstrate a relatively high degree of homing and hence may be managed as separate identities for the purpose of harvesting in these waters. In this sense, management units form a viable stock concept for management of a roe fishery on the spawning grounds while the former population units, or some expansion thereof, would have formed a more appropriate stock concept for management of a reduction fishery on migrating runs.

In this connection, it is interesting to note that the only population in the reduction fishery for which homing was less than 70% (Middle East Coast, 56%) shares a major roe stock (Nanaimo–Comox) with the adjacent lower east coast population (Fig. 1, 3). If these two populations were combined, homing to the resultant single population would be well in excess of 70%.

Another problem in the delineation of populations during the reduction fishery arose from a decision to restrict the location of population boundaries to the use of boundary lines described for the general purpose statistical areas. Consequently, the division between the lower and upper west coast of populations introduced by Stevenson (1954) was made at the boundary between areas 24 and 25 (Fig. 4). Yet, more of the tags applied in the Sydney Inlet management unit in the upper portion of Area 24 were recovered in Area 25 than in Area 24. It would therefore appear that some "fine tuning" of the boundaries between populations, independent of area boundaries, would have resulted in a higher degree of homing to individual populations and hence provided more clear-cut distinctions between them. This form of arbitrary bias has been forestalled in the management unit concept as statistical areas are now subdivided and management units may encompass parts of more than one statistical area (e.g. Chatham Sound, Porcher Island, and Nanaimo–Comox).

Some major inconsistencies remain in the degree of homing estimated for some management units and in the paired estimates based on actual and adjusted recoveries. By expanding the analysis to take into account the effects of differences in the number of years tagged fish were at large (Hourston 1982), changes in the timing and location of fisheries (and hence recovery opportunities) over the years (Hourston and Isaacson 1972), and variations in environmental conditions, it may be possible to account for at least some of these anomalies.

Acknowledgments

The assistance of Debra Lebroq, Lorena Rosenfeld, Fay Winters, Mark Saunders, and others in compiling the data is gratefully acknowledged. Doug Miller prepared the figures. Dan Ware and Carl Haegele provided constructive reviews of the draft manuscript.

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Critique de la méthode de distinction entre poissons anadromes et dulcicoles de la même espèce par la teneur en strontium de leurs écailles

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CASTONGUAY, M., ET G. J. FITZGERALD. 1982. Critique de la méthode de distinction entre poissons anadromes et dulcicoles de la même espèce par la teneur en strontium de leurs écailles. Can. J. Fish. Aquat. Sci. 39: 1423–1425.

Il n'est pas toujours possible de séparer des ombles de fontaine, *Salvelinus fontinalis*, d'eau douce et d'eau salée par la teneur en strontium de leurs écailles parce qu'il arrive que la variabilité intra-groupe soit grande et que la variabilité inter-groupe soit faible. Cette méthode de discrimination entre formes anadromes et dulcicoles ne doit être employée qu'avec prudence. Il semble que l'analyse séparée des différentes parties des écailles d'un omble anadrome ne permette pas de connaître les conditions d'eau douce qu'il a rencontrées antérieurement. La teneur en strontium des écailles des ombles d'eau salée n'a pas diminué suite à un retour en eau douce.

Mots clés: écailles, strontium, salinité, omble de fontaine, anadrome, dulcicole, discrimination

CASTONGUAY, M., ET G. J. FITZGERALD. 1982. Critique de la méthode de distinction entre poissons anadromes et dulcicoles de la même espèce par la teneur en strontium de leurs écailles. Can. J. Fish. Aquat. Sci. 39: 1423–1425.

It was not always possible to distinguish between saltwater and freshwater brook char, *Salvelinus fontinalis*, based on strontium content of scales because of high intra-group variability and low inter-group variability. This method of discrimination between anadromous and freshwater forms should be used with great care. It does not seem possible to determine the freshwater conditions that an anadromous char had previously met by the separate analysis of different parts of the scales. The strontium content of the scales of the saltwater char did not decrease after they were returned to freshwater.

Reçu le 5 mars 1982
Accepté le 25 juin 1982

Received March 5, 1982
Accepted June 25, 1982

La seule méthode existante pour différencier des populations de poissons anadromes et dulcicoles sympatriques de la même espèce est celle mise au point par Bagenal et al. (1973) et reprise par Moreau et Barbeau (1979). Cette méthode est basée sur la plus grande teneur en strontium que présentent les écailles des individus anadromes à cause de la concentration plus élevée en strontium de l'eau de mer. Cependant, certains aspects de la physiologie de l'absorption du strontium dans les écailles soulèvent des doutes quant à la validité de la méthode. Cet article critique ces aspects en répondant aux quatre questions suivantes au moyen d'une expérience réalisée avec l'omble de fontaine, *Salvelinus fontinalis*. (1) Quelle est la variabilité de la concentration en strontium d'écailles provenant d'individus élevés dans des conditions identiques? (2) Est-ce que la détermination d'un seuil de teneur en strontium permet une bonne discrimination entre individus anadromes et dulcicoles? (3) Lorsqu'un individu d'eau douce se retrouve en eau salée, l'augmentation de la teneur en strontium se fait-elle au niveau de l'écaille entière ou seulement dans la zone de croissance produite en eau salée? (4) Se produit-il une baisse du strontium lorsque des individus d'eau salée regagnent l'eau douce?

Matériel et méthodes — L'expérience a été réalisée à l'Aquarium de Québec. Les ombles de fontaine provenaient de la pisciculture de St-Ferréol, Québec et ont été élevés dans des conditions identiques d'eaux douces oligotrophes, typiques du bouclier canadien. Des 12 individus utilisés, 10 étaient âgés de 1+ et avaient une longueur moyenne de 206 mm au début de l'expérience. Les deux autres, âgés de 2+, avaient des longueurs de 305 mm et 313 mm au début. Les concentrations de strontium de ces deux individus n'étaient pas différentes de celles des autres individus pour aucun des prélèvements (test du t, n.s.). Durant l'expérience, la température de l'eau était de 10°C et la photopériode était naturelle. Les poissons furent marqués individuellement avec des étiquettes Floy à double ancrage. Un prélèvement

Printed in Canada (J6833)
Imprimé au Canada (J6833)

TABLEAU 1. Teneur en strontium des écailles des ombles de pisciculture pour les quatre prélèvements effectués.

	Prélèvement			
	Eau douce	Eau salée		Eau douce
	1	2	3	4
Concentration moyenne en strontium (mg/g)[a]	345,6	447,0	493,3	481,1
Ecart-type	91,4	50,8	101,9	59,3
Coefficient de variation corrigé[b]	27,2	11,7	21,6	12,6
Etendu	250–470	370–530	340–610	360–550
Nombre d'individus	9	11	6	9

[a]Une anova ($P < 0.01$) a été calculée sur les données ayant subi une transformation logarithmique. Une comparaison a posteriori « Student–Neuman–Keuls » a ensuite révélé l'existence de deux groupes ($P < 0.01$), un premier groupe formé du prélèvement 1 et un second formé des autres prélèvements.

[b]Voir Sokal et Rohlf (1981).

d'écailles a été effectué au début de l'expérience (prélèvement 1). Les ombles ont ensuite subi une augmentation graduelle de la salinité de 2 ‰ par semaine pour atteindre 20 ‰ après 2,5 mois, puis le prélèvement 2 fut fait. La salinité maximale utilisée était seulement de 20 ‰ parce que dans une tentative précédente où elle avait été montée jusqu'à 30 ‰, les individus avaient subi une forte mortalité. Les ombles furent laissés à la salinité de 20 ‰ durant 4 mois, après quoi d'autres écailles furent prélevées (prélèvement 3). Ils furent ensuite graduellement retournés en eau douce durant 2,5 mois et laissés en eau douce un autre 2,5 mois avant de faire le prélèvement 4. Les poids d'écailles étant parfois insuffisants (0,5 mg est le poids minimal pour doser le strontium selon Moreau et Barbeau (1979)), le nombre d'échantillons analysés varie d'un prélèvement à l'autre (voir tableau 1). La longueur à la fourche de tous les poissons fut mesurée à chaque prélèvement.

Pour des fins de comparaisons, des écailles d'individus anadromes et dulcicoles allopatriques récoltés en nature ont été analysées. Les individus anadromes proviennent de l'estuaire de la rivière St-Jean, Gaspé, Québec et les individus dulcicoles, du lac Ross, un lac du bassin de drainage de la rivière St-Jean. La teneur en strontium a été déterminée par absorption atomique selon une méthode décrite par Moreau et Barbeau (1979).

Résultats et discussion — Même si les coefficients de variation de la teneur en strontium d'écailles d'individus élevés en pisciculture dans des conditions identiques sont grands (tableau 1), l'analyse révèle l'existence de deux groupes: le prélèvement 1 et les autres prélèvements. La concentration moyenne des individus étant en eau salée ou l'ayant fréquenté (prélèvements 2, 3, et 4) est 1,37 fois plus élevée que celle des individus en eau douce (prélèvement 1). Toutefois, quatre individus du prélèvement 1 présentent des concentrations qui recoupent l'étendue des concentrations des autres prélèvements. Le recouvrement entre l'étendue des concentrations du prélèvement 1 et celle des autres prélèvements est de 130 mg/g (340–470 mg/g). Ce recouvrement représente 36,11 % de l'étendue totale qui est de 360 mg/g (250–610 mg/g). Dans ce cas, un seuil de concentration ne permettrait au mieux qu'une distinction partielle entre individus d'eau douce et d'eau salée.

Cependant, on constate chez les poissons récoltés sur le terrain, que le coefficient de variation des poissons d'eau douce (tableau 2) est plus faible que chez les poissons de pisciculture au prélèvement 1 (tableau 1) tandis que le rapport de la concentration moyenne du strontium des individus d'eau salée sur la concentration moyenne des individus d'eau douce est plus grand (1,67 vs. 1,37). Ces différences sont responsables de la meilleure discrimination obtenue chez les poissons récoltés en nature (tableau 2). En effet, une seule valeur de l'étendue des valeurs des individus dulcicoles recoupe l'étendue des valeurs des individus anadromes. Un seuil permettrait donc une juste discrimination de 29 individus sur 30, ce qui est nettement supérieur à ce qui est observé chez les poissons de pisciculture. La qualité de la discrimination d'un seuil sera directement proportionnelle à l'écart entre les moyennes des concentrations des individus dulcicoles et anadromes (variabilité inter-groupe) et inversement proportionnelle à la variabilité intra-groupe. Dans certains cas, un seuil permettra une bonne discrimination et dans d'autres cas, la discrimination ne sera que partielle. Ces résultats, qui ont été obtenus chez l'omble de fontaine, ont des implications plus vastes. Les chercheurs désirant employer cette méthode pour distinguer entre des formes anadromes et dulcicoles doivent réaliser que sa fiabilité n'est que partielle.

Une comparaison effectuée entre les individus dulcicoles en nature (tableau 2) et les individus de la pisciculture au prélèvement 1 (tableau 1) à l'aide d'une anova calculée

TABLEAU 2. Teneur en strontium des écailles des ombles anadromes et dulcicoles récoltés en nature.

	Dulcicoles	Anadromes
Concentration moyenne en strontium (mg/g)[a]	280,3	467,9
Ecart-type	53,7	59,2
Coefficient de variation corrigé	19,3	12,8
Etendue	194–438	382–651
Nombre d'individus	30	29

[a]Différence significative entre individus dulcicoles et anadromes (test du t, $P < 0.01$).

avec le logarithme des valeurs montre une différence significative à $P < 0.001$. Il se produit donc une plus grande déposition de strontium chez les individus de pisciculture qui peut être reliée à la composition chimique des eaux (Moreau et Barbeau 1979) ou à leur diète (Ophel et Judd 1968; Yamada et al. 1979). Par contre, les valeurs des ombles de pisciculture en eau salée (tableau 1) et des ombles anadromes en nature (tableau 2) ne sont pas statistiquement différentes (anova, n.s.).

L'incorporation du strontium additionnel, causée par l'augmentation de la salinité chez les individus de pisciculture, est terminée après 2,5 mois, car la concentration du strontium ne varie pas significativement à partir du 2ième prélèvement (tableau 1). La croissance moyenne en longueur des poissons entre le premier et le deuxième prélèvement est de 11,4 %. On peut supposer que la croissance des écailles pour la même période est voisine de cette valeur. Pour que l'augmentation de la teneur en strontium des écailles entières, observée entre le premier et le deuxième prélèvement, soit uniquement causée par une incorporation plus grande de la portion des écailles produite durant cette période, il faudrait que celle-ci présente une teneur moyenne de 1245,6 mg/g de strontium. Cette valeur est élevée par rapport à ce que nous avons obtenu chez les individus anadromes en nature et ce que d'autres auteurs ont trouvé chez d'autres espèces (Bagenal et al. 1973; Moreau et Barbeau 1979). Il semble donc que l'augmentation observée entre le premier et le deuxième prélèvement est causée par une incorporation de l'écaille entière. Ceci implique un changement des concentrations de toutes les parties de l'écaille lorsqu'un omble passe de l'eau douce à l'eau salée. L'analyse séparée des différentes parties de l'écaille ne permet donc pas de reconnaître les conditions d'eau douce que le poisson capturé en eau salée a rencontrées antérieurement. Malheureusement, il n'a pas été possible de vérifier cela directement parce que les écailles d'ombles de fontaine sont trop petites pour être analysées selon leurs différentes parties avec la technique utilisée. D'autre part, Bagenal et al. (1973) ont trouvé que la portion d'écailles de deux saumons atlantiques (*Salmo salar*) correspondant à la vie en eau douce présentait des concentrations plus faibles que la portion produite en eau salée.

S'il se produisait une remise en circulation du strontium des écailles lorsque les individus sont retournés en eau douce, une baisse de la concentration de strontium aurait été observée. Le tableau 1 montre que celle-ci ne s'est pas produite. Le strontium est donc resté fixé dans les écailles, 2,5 mois après un retour en eau douce. Bagenal et al. (1973) ont obtenu un résultat similaire chez la truite brune (*Salmo trutta*), après 67 jours en eau douce. De plus, il semble que les concentrations restent stables à long terme. En effet, Ophel et Judd (1968), ayant montré qu'une diète riche en strontium provoquait une forte augmentation de la teneur en strontium des écailles chez le poisson doré (*Carassius auratus*), ont déterminé que la teneur en strontium est restée stable à 10 fois la concentration du groupe témoin, un an après que les poissons aient repris une diète normale. Yamada et al. (1979) ont aussi déterminé que la diminution du strontium de la portion de vertèbres de saumon coho (*Oncorhynchus kisutch*) produite durant une période de diète riche en strontium est faible après 2 ans. Il semble donc que la concentration en strontium de la portion des écailles produite en eau salée ne diminue pas lors d'un retour en eau douce.

Remerciements — Nous remercions le personnel de l'Aquarium de Québec et du département de chimie de l'Université Laval pour leur grande contribution à cette étude. Les fonds qui ont soutenu cette recherche proviennent de la Faculté des sciences et de génie de l'Université Laval. Les Drs J. Dodson, G. Moreau et C. Cloutier ont apporté leurs critiques à ce manuscrit. Le Dr Leggett et un arbitre anonyme ont commenté une version ultérieure.

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Acute Toxicity of Cyanide to Rainbow Trout (*Salmo gairdneri*) Acclimated at Different Temperatures

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KOVACS, T. G., AND G. LEDUC. 1982. Acute toxicity of cyanide to rainbow trout (*Salmo gairdneri*) acclimated at different temperatures. Can. J. Fish. Aquat. Sci. 39: 1426 – 1429.

The toxicity of cyanide (HCN) to juvenile rainbow trout (*Salmo gairdneri*) acclimated for 3 wk at 6, 12, and 18°C was determined at these temperatures by flow-through bioassays, in the cyanide concentration range of 0.018−0.087 mg · L^{-1}. The 96-h median lethal concentrations (LC50) were 0.028 ± 0.004 mg · L^{-1} at 6°C, 0.042 ± 0.004 mg · L^{-1} at 12°C, and 0.068 ± 0.004 mg · L^{-1} at 18°C. Warm acclimated rainbow trout survived longer in lethal concentrations of cyanide. Toxicity curves clearly showed the temperature effect on the acute toxicity of cyanide is concentration dependent. At slowly lethal concentrations, cyanide is more toxic at lower temperatures, whereas at rapidly lethal levels the reverse occurs; the reversal takes place at 0.10 mg · L^{-1} HCN.

Key words: cyanide, acute toxicity, temperature, rainbow trout

KOVACS, T. G., AND G. LEDUC. 1982. Acute toxicity of cyanide to rainbow trout (*Salmo gairdneri*) acclimated at different temperatures. Can. J. Fish. Aquat. Sci. 39: 1426 – 1429.

La toxicité du cyanure (HCN) a été mesurée sur la truite arc-en-ciel (*Salmo gairdneri*) acclimatée durant 3 sem à 6, 12, et 18°C. Les dosages biologiques ont été effectués à débit continu sur des concentrations de cyanure variant de 0,018 à 0,087 mg · L^{-1} HCN. Les concentrations médianes létales (LC50) déterminées furent les suivantes: 0,028 ± 0,004 mg · L^{-1} à 6°C, 0,042 ± 0,004 mg · L^{-1} à 12°C et 0,068 ± 0,004 mg · L^{-1} HCN à 18°C. Les truites acclimatées aux températures élevées ont survécu plus longtemps que celles en eau froide. Les courbes de toxicité indiquent que la toxicité aiguë du cyanure à différentes températures dépend de la concentration. Aux concentrations lentement létales le cyanure est plus toxique aux basses températures mais le contraire se produit aux concentrations rapidement létales. Le changement d'effet de température sur la toxicité se produit à 0,10 mg · L^{-1} HCN.

Received December 16, 1980
Accepted June 18, 1982

Reçu le 16 décembre 1980
Accepté le 18 juin 1982

TEMPERATURE stress, as a modifying factor of toxicity, has been given only little attention in establishing water quality criteria for toxic substances. The effects of temperature on toxicity have been reviewed by Cairns et al. (1975) but no generalizations can be made because published information is scanty, inconclusive, and sometimes contradictory. The purpose of our study was to evaluate the acute toxicity of cyanide to rainbow trout (*Salmo gairdneri*) acclimated and tested at three different temperatures.

Cyanide toxicity to fish has been studied under various experimental conditions, with many species of fish (see reviews by Doudoroff 1976 and 1980). The available literature suggests lethal threshold concentrations of 0.05−0.07 mg · L^{-1} HCN for salmonids tested at 15−17°C (Herbert and Merkens 1952; Burdick et al. 1958; Brown 1968), whereas the lethal threshold for warmwater fishes (20−25°C) lies in the range of 0.10−0.15 mg · L^{-1} HCN (Burdick et al. 1958; Doudoroff et al. 1966).

Temperature effects on cyanide toxicity to trout were first observed by Southgate et al. (1932), Alexander et al. (1935), Wuhrmann and Woker (1955), and Cairns and Scheier (1963), who showed that higher temperatures increased toxicity. On the other hand, researchers from Great Britain (in Doudoroff 1976) found that at slowly lethal concentrations cyanide was more toxic to trout at low temperatures (3−4°C). The toxicity of cyanide to different species of freshwater fishes tested under different environmental conditions was reported by Smith et al. (1978). Higher temperatures seemed to reduce the toxicity of cyanide to the species tested, but tem-

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Printed in Canada (J6329)
Imprimé au Canada (J6329)

TABLE 1. Acute toxicity of hydrogen cyanide to rainbow trout acclimated and tested at different temperatures in flow-through system for 96 h.

	6°C	12°C	18°C
96-h LC50[a] (95% confidence interval[b]), mg · L^{-1} HCN	0.028 (0.024–0.035)	0.042 (0.038–0.046)	0.068 (0.064–0.072)
Slope function	1.30	1.30	1.12
Highest cyanide concn. with no mortality in 96 h, mg · L^{-1} HCN	0.018	0.032	0.060
Lowest cyanide concn. with 100% mortality in 96 h, mg · L^{-1} HCN	0.037	0.053	0.087
Average weight of fish (range), g	11.79 (9.05–15.98)	12.41 (8.29–16.02)	13.19 (9.63–17.21)
Average fork length of fish (range), mm	102 (95–110)	102 (90–111)	106 (95–119)
Mean pH (range)	8.06 (7.9–8.1)	8.10 (8.06–8.11)	7.82 (7.78–7.90)
Mean oxygen saturation (range), %	89.2 (88.2–90.1)	97.1 (95.2–98.5)	86.8 (85.7–88.3)

[a]Determined from log-probit paper.
[b]Litchfield and Wilcoxon (1949).

perature modification of cyanide toxicity to rainbow trout was not determined. These divergent conclusions seemed to arise from different ranges of concentrations tested and insufficient thermal acclimation.

Material and methods — Rainbow trout purchased from La Pisciculture, Mt. Sutton, Sutton (Qué.), were held in 200-L fiberglass oval-shaped tanks at a temperature of 12 ± 1°C. Chemical characteristics of the dechlorinated Montreal city water used during the experiments (December 1976 to July 1977) were as follows: alkalinity, 87 mg · L^{-1} as $CaCO_3$; hardness, 127 mg · L^{-1} as $CaCO_3$; CO_2, 0.47 mg · L^{-1}. See Table 1 for oxygen and pH levels.

After at least 2 wk acclimation in the 200-L holding tanks at 12°C, the fish to be tested at 6 and 18°C were segregated and acclimated to the desired water temperatures by gradual adjustments of about 1°C · d^{-1}. The fish were then held for 7 d at these temperatures, prior to transfer into the white polyethylene (68 × 57.5 × 42 cm deep) test tanks, where they were held a further 2 wk at the test temperatures (i.e. acclimated for 3 wk at test temperatures). The tanks were connected to a flow-through system provided with thermally controlled water, at a flow of 1 L · min^{-1} in each tank, giving 99% replacement in 6 h, as calculated after Sprague (1973). The entire 24-tank assembly was illuminated evenly by fluorescent lights, controlled by a time switch to provide a 12-h photoperiod (08:30–20:30). During this period, the fish were fed daily but no food was given for 48 h before the tests.

The acute toxicity of HCN to rainbow trout at 6, 12, and 18°C was determined according to Sprague (1973). At hour zero, a calculated amount of cyanide stock solution was mixed into each tank to produce immediately the predicted concentrations which were then maintained by Mariotte bottles (Leduc 1966). Cyanide was monitored in the test tanks twice daily, according to Lambert et al. (1975). The measured concentrations never differed by more than 1% of the predicted values.

The concentrations tested (mg · L^{-1} HCN) ranged from 0.018 to 0.056 at 6°C, 0.032 to 0.087 at 12°C, and 0.042 to 0.087 at 18°C. These ranges were selected from preliminary tests and included concentrations causing zero and 100% mortality. Control groups were maintained at each temperature under identical conditions.

Results — The resistance of juvenile rainbow trout to cyanide was markedly affected by temperature (Table 1) and each 96-h LC50 value differed significantly ($P < 0.05$, tested by the method of Litchfield and Wilcoxon (1949)) from those obtained at other temperatures. The cyanide level required to kill 50% of the trout in 96 h was 2.4 times lower at 6 than at 18°C.

The median survival times (MST) for each test concentration were estimated following Sprague (1973). Eye-fitted toxicity curves were constructed over cyanide concentrations ranging from 0.028 to 1.0 mg · L^{-1}, using data from this study and comparable data from the literature (Ministry of Technology 1968; Herbert and Merkens 1952). At concentrations below 0.10 mg · L^{-1} HCN, the MST decreased at a lower temperature (Fig. 1). The lethal threshold was reached in 96 h at 18°C but not at 12 and 6°C. As the concentrations tested increased, the difference in the MST at each temperature became smaller (Fig. 1). Indeed, the curves intersect

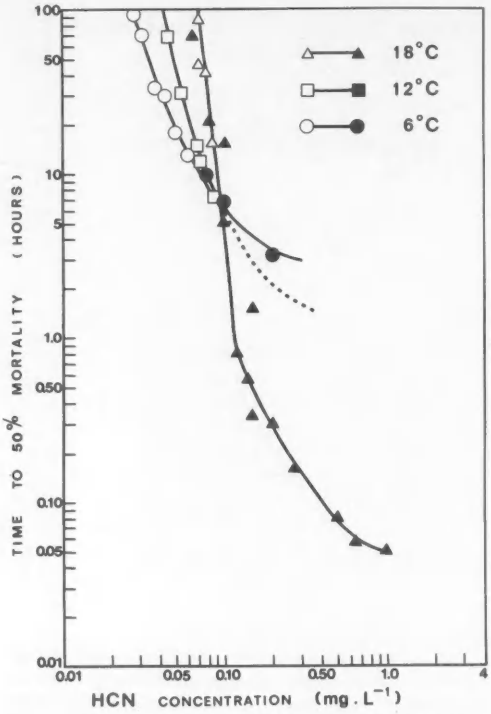

FIG. 1. Toxicity of hydrogen cyanide to rainbow trout at three temperatures: this study (open symbols); Herbert and Merkens (1952) (closed triangles); Ministry of Technology (1968) (closed circles).

near 0.1 mg · L^{-1} HCN, above which the temperature effect on the lethal action of cyanide to trout is reversed. Thus at more rapidly lethal concentrations cyanide becomes more toxic at 18°C than at 12 and 6°C.

Discussion — Toxicological significance — Our results agree with only some previous reports. Our 96-h LC50 values at 6 and 18°C, respectively, compare to the median threshold concentrations reported for similar temperatures by the Ministry of Technology (1968) and by Herbert and Merkens (1952). On the contrary Southgate et al. (1932) and Alexander et al. (1935) found that toxicity of cyanide to trout increased with temperature. Smith et al. (1978) also reported that the toxicity of cyanide to several species of fish (excluding rainbow trout) decreased at higher temperatures. The apparent contradictions in the earlier and more recent literature seem to be resolved here by the comparison of the toxicity curves at 6, 12, and 18°C (see Fig. 1). This figure confirms that the potency of cyanide at low and high temperatures is concentration dependent.

Matida (1960) pointed out that physiological and biochemical processes leading to death may be different in short-term exposure to high concentrations of a toxicant from those in long-term exposure to more dilute solutions. At high concentrations of HCN, death probably occurs because of the disruption in the activity of vital organs such as the heart and central nervous system (Jones 1964). Time to reach the reaction site (induction time) depends on the rate of uptake of the toxicant and its distribution to the tissues. Higher temperatures increase the ventilation rate of fish (Heath 1973) and toxicant uptake from the water at the gills, and promote a faster distribution to the reaction site(s).

At lower concentrations, death from HCN may reflect the inhibition of the cytochrome chain in the whole animal. In fish, different temperature-related biochemical makeup at the cellular and/or metabolic levels are known (Hochachka and Somero 1973) and would undoubtedly alter sensitivity to cyanide. Ekberg (1958) found the gills of the goldfish (*Carassuis auratus*) more resistant to cyanide at 30 than at 10°C and attributed this to a temperature-related qualitative change of cytochrome oxidase, (Ekberg 1962). At low lethal concentrations, cyanide detoxification by the enzyme rhodanese (thiosulphate sulphurtransferase, EC 2.8.1.1) (Schievelbein et al. 1969; Sido and Koj 1972) may also play an important role. It is reasonable to assume that the activity of rhodanese would also be temperature related.

Physiological significance — The differential effect of temperature at rapidly and slowly lethal concentrations to fish also occurs with other toxicants. Lethal thresholds of zinc concentrations were higher at 11 and 19°C than at 3°C when Atlantic salmon (*Salmo salar*) were tested in 14-d bioassays (Hodson and Sprague 1975). With high concentrations of phenol, Brown et al. (1967) observed increased mortality of rainbow trout at higher temperatures and a reversed reaction when lower concentrations were tested. With ammonia, Lloyd and Herbert (1962) and Lloyd (1961) found no effect of temperature on the lethal threshold, but Brown (1968), testing higher concentrations, found the 48-h LC50 to decrease with an increasing temperature. Similar results were obtained by Smith and Heath (1979) using five species of fish to test the acute toxicity of cyanide, chromate, copper, and zinc at three temperatures.

The prediction of acceptable concentrations of chemicals for aquatic organisms has and will remain the ultimate goal of water pollution research. This constant need to estimate safe levels is amplified by the ever-increasing number of new chemicals each year, for which short-term acute toxicity data must often be substituted for the slowly acquired and costly chronic data. The lethal test remains the main source of information for the aquatic toxicologist on which to base a scientific judgment on the hazard of a toxicant in nature. The notion of application factor, or a safe dilution of the LC50, must remain biologically valid (Mount 1977). We have shown a concentration dependence of the effects of temperature on cyanide toxicity to trout and this phenomenon should therefore be considered whenever application factors are derived solely from LC50 data in the context of varying temperature due to seasonal and/or geographical factors.

Acknowledgments — This project was supported by a Department of Indian and Northern Affairs research contract (OSU5-0010). The Department of Education of the Province of Quebec also provided financial assistance through a graduate scholarship.

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Comment on "Multivariate Analysis as a Means of Comparing Growth in Fish"

COMMENT

In a recent issue of this journal (*Can. J. Fish. Aquat. Sci.* 38: 233–236), D. R. Bernard (1981) discussed the application of the Hotelling's T^2 method to growth data to test for significant differences between parameter estimates obtained by fitting (by least squares) von Bertalanffy curves to two groups of fish. Despite the apparent attractiveness of the T^2 method for comparing growth parameters between two groups of fish, we feel that it is inappropriate for this purpose for many reasons.

Growth data can be classified into the general class of designs, extensively studied under the heading of Analysis of Covariance (ANCOVA), where one has a data set consisting of observations from an independent variable (age) and a dependent variable (length) partitioned into "between" groups (ages) and "within" groups. This type of analysis yields two types of regression: within groups and between groups. (For a more complete discussion of this subject see Snedecor and Cochran (1967) p. 436–438.) However, for the type of data sets to which the von Bertalanffy curve is fitted, the values for the independent variable are constant within groups and therefore there can be no "within" group regression. One consequence of this is that the number of degrees of freedom for the T^2 test, in fact the related F test, as presented by Bernard is incorrect. Bernard's N_1 and N_2 should equal the number of age-groups and not the total number of fish in each sample. A second consequence is that Bernard's use of Ito and Schull's (1964) conclusions as to the robustness of T^2 when the variance–covariance matrices from the two samples are not equal are not applicable; not only must $N_1 = N_2$ hold for these results to be true, but also N_1 and N_2 must be large in value.

In addition, there is the problem of unequal between-age variances of the length measurements. In growth data obtained from fish it has been noted that the standard deviation of length measurements tend to vary systematically with age often in a curvilinear pattern (Ricker 1975, p. 211; Iles 1980; Misra 1980, fig. 2). Apart from the fact that the variances of the lengths may change with age, heterogeneity of variance occurs if the number of length measurements in each age-group are not equal (Steele and Torrie 1960, p. 181). In his analysis of the Pacific ocean perch (*Sebastes alutus*) data, Bernard set the sample sizes equal by randomly sampling a set number of observations from the original data. This may avoid the second source of heterogeneity mentioned above, but it results in a loss of information. The natural solution to this problem of unequal variances is to use a weighted procedure such as is presented in Misra (1980).

As the computer programs of Tomlinson and Abramson (1961) used by Bernard to estimate the growth parameters assume homogeneity of variances, the resulting variance–covariance matrices are suspect.

Testing differences between parameter estimates by the T^2 method when heterogeneity is present is an example of the Behrens–Fisher problem for the multivariate case (see Snedecor and Cochran 1967, p. 115). At present only approximate procedures are available to deal with this problem, and we refer the reader to Misra (1980) for one such technique. — R. K. MISRA, *Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7* AND S. J. SMITH, *Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2.*

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REPLY

The criticisms by Misra and Smith, even if valid, do not warrant the conclusion that the Hotelling's T^2 is an inappropriate test for discriminating differences in growth between groups of fish. All of Misra's and Smith's comments concern either the degrees of freedom or the possible biases in the variance–covariance matrices used in the tests in Bernard (1981); their comments relate not to the propriety of the T^2 test, but to its proper application. The Hotelling T^2 is a multivariate test of statistical hypotheses that involve sets of parameters, and it is the appropriate test whenever the parameters are correlated within each set (the magnitude of off-diagonal elements in the correlation matrix for each set are close to unity) (see Cooley and Lohnes 1971, p. 3–14; Morrison 1976, p. 80–90); if parameters are not correlated within sets (the magnitude of off-diagonal elements in the correlation matrix for each set are near zero), then Student's t (Draper and Smith 1981) or ANCOVA (Snedecor and Cochran 1967) on individual comparisons of parameters between sets are the appropriate tests. On the condition of the correlation matrix, and on this condition alone, is the T^2 an appropriate or an inappropriate statistic.

The degrees of freedom used in the T^2 tests in Bernard

Printed in Canada (J6743a)
Imprimé au Canada (J6743a)

(1981) reflect the repeated measurements of dependent variables for each value of the independent variable in the regression, which is a valid approach to regression (Draper and Smith 1982, p. 33–45). However, the number of degrees of freedom in the T^2 (or in any other statistical test) is relevant to its application, not to its appropriateness.

Large degrees of freedom are a preferred condition for the T^2 statistic, and to all other nonparametric and parametric statistical tests as well. Equal numbers of measurements in each group of fish remove the biases caused by different variance–covariance matrices in the Hotelling's T^2 (Ito and Schull 1964); large numbers of fish are needed to reveal better the underlying probability distributions, not to ensure a normal probability distribution for the test.

When fitting a model like the von Bertalanffy growth curve to length data with heterogeneous conditional distributions on age, a weighted regression should be used; Misra and Smith are correct. However, a weighted regression is necessary only when the conditional variances of length given age are significantly different; in the data from Bernard (1981), these variances are not. Even though most stocks have different conditional variances of length at age as cited in Misra and Smith (1982), enough of the life span must be included in an analysis for this difference to cause a significant bias and warrant a weighted regression. In Bernard (1981), Bartlett's test for homogeneity of variances showed the conditional variances not significantly different over the years represented in the data. Considering the slow growth of Pacific ocean perch (*Sebastes alutus*) and the relatively short span of ages in the analysis (6–18 yr), this lack of significance is not surprising. However, whenever conditional variances are different, a weighted fitting procedure should be used.

Finally, I do not believe in ignoring useful information, but neither do I believe in using information when it is redundant. A cursory glance at most statistical tables like those for the F and the t distributions shows that each additional degree of freedom represents a smaller marginal amount of information in a test. Not using degrees of freedom beyond the number needed to describe adequately a distribution is not a loss of information, but is a gain in efficiency. — DAVID R. BERNARD, *Stock Separation Group, Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99502, USA.*

BERNARD, D. R. 1981. Multivariate analysis as a means of comparing growth in fish. Can. J. Fish. Aquat. Sci. 38: 233–236.

COOLEY, W. W., AND P. R. LOHNES. 1971. Multivariate data analysis. John Wiley and Sons, Inc., New York, NY. 364 p.

DRAPER, N. R., AND H. SMITH. 1981. Applied regression analysis, 2nd ed. John Wiley and Sons, Inc., New York, NY. 709 p.

ITO, K., AND W. J. SCHULL. 1964. On the robustness of the T_0^2 test in multivariate analysis of variance when variance–covariance matrices are not equal. Biometrika 51: 71–72.

MORRISON, D. F. 1976. Multivariate statistical methods. 2nd ed. McGraw-Hill Book Company, New York, NY. 415 p.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods. 6th ed. The Iowa State University Press, Ames, IA. 593 p.

Printed in Canada (J6743b)
Imprimé au Canada (J6743b)

Books Received/Livres reçus[1]

June/juin 1982

Marine Fisheries Ecosystem: its quantitative evaluation and management. *Taivo Laevastu and Herbert A. Larkins. Fishing News Books Ltd., Farnham, Surrey, England. 1981. 162 p. £15.50 + £1.55 post/packing.* This book summarizes in quantitative terms the major processes of a marine ecosystem while emphasizing fish biota and its relation to the environment. The study outlines a quantitative approach for the computerized evaluation of the marine fish ecosystem which is suitable for the evaluation of fishery resources and for their modern management. [R436]

Renewable Resources in the Pacific. Proceedings of the 12th Pacific Trade and Development Conference, held in Vancouver, Canada, 7–11 September 1981. *Edited by H. E. English and Anthony Scott. International Development Research Centre, Ottawa, Canada. 1982. 293 p. $15.* The conference's focus was renewable resources and economic issues. The relationship between trade policies and changing comparative advantage in the developing countries of east and southeast Asia was examined. The major subjects discussed were fisheries, forests, and energy. [R437]

The Biology and Culture of Tilapias. *Edited by R. S. V. Pullin and R. H. Lowe-McConnell. International Center for Living Aquatic Resources Management (ICLARM), Metro Manilla, Philippines. ICLARM Conference Proceedings 7. 1982. 432 p. U.S. $13. surface mail soft cover, $17.50 surface hard cover, $25. airmail soft cover, $29.50 airmail hard cover.* Proceedings of the International Conference on the Biology and Culture of Tilapias, September 2–5, 1980, at the Study and Conference Center of the Rockefeller Foundation, Bellagro, Italy. The 15 comprehensive review papers together with summaries of the discussions on the papers, as well as concensus statements on research and information, cover tilapia biology, physiology, and culture-related topics. Participants addressed issues ranging from standardization of nomenclature and techniques for the development of known genetic strains and hybrids to optimization of growth and feeding and control of reproduction. [R438]

Les crabes d'eau douce d'Amérique : Famille des Pseudothelphusidae. *Gilberto Rodriguez. L'Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM) Paris. 1982. Faune Tropicale 22. 223 p.* Les crabes d'eau douce représentent un élément faunistique important dans les eaux continentales de l'Amérique tropicale. Les Pseudothelphusidae vivent dans les ruisseaux de montagne jusqu'à 3000 m d'altitude. L'auteur nous offre un ouvrage qui facilitera considérablement leur identification. [R439]

La pêche des thons à la senne et à la canne dans l'Océan Pacifique tropical : situation actuelle et perspectives de développement. *J. Marcille et W. Bour, L'Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), Paris. 1981. Travaux et Documents de l'ORSTOM 34. 259 p.* Après un bref aperçu de la biologie des deux principales espèces listao et albacore, les auteurs examinent la situation actuelle de la pêche à la senne et à la canne et présentent un schéma possible d'exploitation du Pacifique Sud-Ouest et Sud-Central. [R440]

Milieu marin et ressources halieutiques de la République Populaire du Congo. *Éditeur scientifique, André Fontana. L'Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), Paris. 1981. Travaux et Documents de l'ORSTOM 138. 339 p.* La première partie de l'ouvrage est consacrée à l'étude du milieu marin (sédimentologie, hydro-climatologie, productivité primaire et secondaire du plancton) et la seconde partie à la revue des différents stocks et des schémas d'aménagement préconisés par l'ORSTOM. [R441]

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[1]Persons interested in reviewing books in this list should contact the Book Review Editor, Canadian Journal of Fisheries and Aquatic Sciences, Scientific Information and Publications Branch, Department of Fisheries and Oceans, Ottawa, Canada K1A 0E6.

[1]Les personnes intéressées à examiner les livres énumérés dans cette liste sont priées de communiquer avec le Rédacteur des comptes rendus, Journal canadien des sciences halieutiques et aquatiques, Direction de l'information et des publications scientifiques, Ottawa, Canada K1A 0E6.

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Empirical estimates of carbon production for a meiobenthic harpacticoid copepod **R. J. Feller**

Model of salmon age structure and its use in preseason forecasting and studies of marine survival **R. M. Peterman**

Release of methyl mercury from sediments: effects of mercury concentration, low temperature, and nutrient addition **D. R. Wright and R. D. Hamilton**

Stock Reduction Analysis, another solution to the catch equations **D. K. Kimura and J. V. Tagart**

Descriptions and keys for ammocoetes of lampreys from British Columbia, Canada **J. E. Richards, R. J. Beamish, and F. W. H. Beamish**

Immunogenic potential of growth products extracted from cultures of *Aeromonas salmonicida* for brook trout (*Salvelinus fontinalis*) **R. C. Cipriano**

Competitive interactions between benthic stream fishes, riffle sculpin, *Cottus gulosus*, and speckled dace, *Rhinichthys osculus* **D. M. Baltz, P. B. Moyle, and N. J. Knight**

Trophic upsurge in new reservoirs: a model for total phosphorus concentrations **Y. Grimard and H. G. Jones**

Stock–recruitment relationship for Atlantic salmon (*Salmo salar*) in Newfoundland rivers **E. M. P. Chadwick**

Influence of hatchery experience on growth and behavior of juvenile Atlantic salmon (*Salmo salar*) within allopatric and sympatric stream populations **T. A. Dickson and H. R. MacCrimmon**

Histology and prevalence of X-cell lesions in Atlantic cod (*Gadus morhua*) **C. M. Morrison, G. Shum, R. G. Appy, P. Odense, and C. Annand**

Notes

A multilocus genetic model applied to the effects of selective fishing on the growth rate of trout **L. D. Favro, P. K. Kuo, J. F. McDonald, D. D. Favro, and A. D. Kuo**

Predation by medusae on Pacific herring (*Clupea harengus pallasi*) larvae **M. N. Arai and D. E. Hay**

Effects of upwelling on local abundance and distribution of larval alewife (*Alosa pseudoharengus*) in eastern Lake Michigan **G. R. Heufelder, D. J. Jude, and F. J. Tesar**

Letters and Comments/Lettres et commentaires: Comment on electroencephalographic responses to morpholine and their relationship to homing **J. Cooper;** Reply **T. J. Hara and S. B. Brown**

Obituary/Nécrologie — Professor Michitaka Uda, 1905–1982 **T. R. Parsons**

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

CJFSDX39(10) 1335–1433 (1982)
ISSN 0706-652X

Volume 39, No. 10, October 1982 Volume 39, n° 10, octobre 1982

Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry **K. H. Wilson and P. A. Larkin** 1335–1339

Otoliths of chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production **J. D. Neilson and G. H. Geen** 1340–1347

Carbon inputs to bivalve mollusks: a comparison of two estuaries **L. S. Incze, L. M. Mayer, E. B. Sherr, and S. A. Macko** 1348–1352

Spawning behavior and early life history of pink salmon (*Oncorhynchus gorbuscha*) in the Great Lakes **W. Kwain** 1353–1360

A model for predicting Pacific herring (*Clupea harengus pallasi*) spawn density from diver observations **J. F. Schweigert and D. Fournier** 1361–1365

Temperature and scale size errors in the use of [^{14}C]glycine uptake by scales as a growth index **C. M. Smagula and I. R. Adelman** 1366–1372

Problems of species identity in the Lake Ontario sculpins *Cottus bairdi* and *C. cognatus* **C. M. Godkin, W. J. Christie, and D. E. McAllister** 1373–1382

An evaluation of the wet oxidation technique for use in determining the energy content of seston samples **R. I. E. Newell** 1383–1388

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Homing by Canada's west coast herring to management units and divisions as indicated by tag recoveries **A. S. Hourston** 1414–1422

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Acute toxicity of cyanide to rainbow trout (*Salmo gairdneri*) acclimated at different temperatures **T. G. Kovacs and G. Leduc** 1426–1429

Letters and comments/Lettres et commentaires: Comment on "Multivariate analysis as a means of comparing growth in fish" **R. K. Misra and S. J. Smith;** Reply **D. R. Bernard** 1430–1431

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